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(54) Title: 52 HUMAN SECRETED PROTEINS

(57) Abstract: The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating diseases, disorders, and/or conditions related to these novel human secreted proteins.

52 Human Secreted Proteins

Field of the Invention

This invention relates to newly identified polynucleotides, polypeptides encoded by these polynucleotides, antibodies that bind these polypeptides, uses of such polynucleotides, polypeptides, and antibodies, and their production.

Background of the Invention

Unlike bacterium, which exist as a single compartment surrounded by a membrane, human cells and other eucaryotes are subdivided by membranes into many functionally distinct compartments. Each membrane-bounded compartment, or organelle, contains different proteins essential for the function of the organelle. The cell uses "sorting signals," which are amino acid motifs located within the protein, to target proteins to particular cellular organelles.

One type of sorting signal, called a signal sequence, a signal peptide, or a leader sequence, directs a class of proteins to an organelle called the endoplasmic reticulum (ER). The ER separates the membrane-bounded proteins from all other types of proteins. Once localized to the ER, both groups of proteins can be further directed to another organelle called the Golgi apparatus. Here, the Golgi distributes the proteins to vesicles, including secretory vesicles, the cell membrane, lysosomes, and the other organelles.

Proteins targeted to the ER by a signal sequence can be released into the extracellular space as a secreted protein. For example, vesicles containing secreted proteins can fuse with the cell membrane and release their contents into the extracellular space - a process called exocytosis. Exocytosis can occur constitutively or after receipt of a triggering signal. In the latter case, the proteins are stored in secretory vesicles (or secretory granules) until exocytosis is triggered. Similarly, proteins residing on the cell membrane can also be secreted into the extracellular space by proteolytic cleavage of a "linker" holding the protein to the membrane.

Despite the great progress made in recent years, only a small number of genes encoding human secreted proteins have been identified. These secreted proteins include the commercially valuable human insulin, interferon, Factor VIII, human growth hormone, tissue plasminogen activator, and erythropoeitin. Thus, in light of

the pervasive role of secreted proteins in human physiology, a need exists for identifying and characterizing novel human secreted proteins and the genes that encode them. This knowledge will allow one to detect, to treat, and to prevent medical diseases, disorders, and/or conditions by using secreted proteins or the genes that encode them.

Summary of the Invention

The present invention relates to novel polynucleotides and the encoded polypeptides. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant and synthetic methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the polypeptides and polynucleotides, and therapeutic methods for treating such diseases, disorders, and/or conditions. The invention further relates to screening methods for identifying binding partners of the polypeptides.

Detailed Description

Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

In the present invention, a "secreted" protein refers to those proteins capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5 kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X or the cDNA contained within the clone deposited with the ATCC. For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

In the present invention, the full length sequence identified as SEQ ID NO:X was often generated by overlapping sequences contained in multiple clones (contig analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X was deposited with the American Type Culture Collection ("ATCC"). As

shown in Table 1, each clone is identified by a cDNA Clone ID (Identifier) and the ATCC Deposit Number. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:X, the complement thereof, or the cDNA within the clone deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH_2PO_4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched , for example, as a result of

ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

"SEQ ID NO:X" refers to a polynucleotide sequence while "SEQ ID NO:Y" refers to a polypeptide sequence, both sequences identified by an integer specified in Table 1.

"A polypeptide having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention.)

Many proteins (and translated DNA sequences) contain regions where the amino acid composition is highly biased toward a small subset of the available

residues. For example, membrane spanning domains and signal peptides (which are also membrane spanning) typically contain long stretches where Leucine (L), Valine (V), Alanine (A), and Isoleucine (I) predominate. Poly-Adenosine tracts (polyA) at the end of cDNAs appear in forward translations as poly-Lysine (poly-K) and poly-Phenylalanine (poly-F) when the reverse complement is translated. These regions are often referred to as "low complexity" regions.

Such regions can cause database similarity search programs such as BLAST to find high-scoring sequence matches that do not imply true homology. The problem is exacerbated by the fact that most weight matrices (used to score the alignments generated by BLAST) give a match between any of a group of hydrophobic amino acids (L,V and I) that are commonly found in certain low complexity regions almost as high a score as for exact matches.

In order to compensate for this, BLASTX.2 (version 2.0a5MP-WashU) employs two filters ("seg" and "xnu") which "mask" the low complexity regions in a particular sequence. These filters parse the sequence for such regions, and create a new sequence in which the amino acids in the low complexity region have been replaced with the character "X". This is then used as the input sequence (sometimes referred to herein as "Query" and/or "Q") to the BLASTX program. While this regime helps to ensure that high-scoring matches represent true homology, there is a negative consequence in that the BLASTX program uses the query sequence that has been masked by the filters to draw alignments.

Thus, a stretch of "X"s in an alignment shown in the following application does not necessarily indicate that either the underlying DNA sequence or the translated protein sequence is unknown or uncertain. Nor is the presence of such stretches meant to indicate that the sequence is identical or not identical to the sequence disclosed in the alignment of the present invention. Such stretches may simply indicate that the BLASTX program masked amino acids in that region due to the detection of a low complexity region, as defined above. In all cases, the reference sequence(s) (sometimes referred to herein as "Subject", "Sbjct", and/or "S") indicated in the specification, sequence table (Table 1), and/or the deposited clone is (are) the definitive embodiment(s) of the present invention, and should not be construed as

limiting the present invention to the partial sequence shown in an alignment, unless specifically noted otherwise herein.

Polynucleotides and Polypeptides of the Invention

FEATURES OF PROTEIN ENCODED BY GENE NO: 1

The translation product of this gene shares sequence homology with env protein (see, e.g., Genbank accession number AAD34324.1 (AF108843); all references available through this accession are hereby incorporated by reference herein.), a protein with similarity to retroviral envelope glycoproteins.

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 493 to about 509 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing from about amino acids 510 to about 563 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ia membrane proteins.

This gene is expressed primarily in fetal tissues, placenta, fetal liver spleen, infant brain, and total fetus and to a lesser extent in tumors (poorly differentiated ovarian adenocarcinoma and endometrial tumor), human adult (K.Okubo) and PC3 prostate cell line.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: fetal development disorders, cancer and other proliferative disorders, particularly endometrial and ovarian cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endometrium and ovary, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell

types (e.g., fetal, reproductive, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 83 as residues: Gln-88 to Lys-97, Glu-128 to Ser-133, Asn-166 to Pro-175, Thr-191 to Asn-196, Asn-207 to Lys-212, Cys-232 to Gly-238, Ala-256 to Ala-263, Thr-268 to Thr-280, Pro-311 to Cys-317, Val-347 to Leu-362, Glu-396 to Leu-406, Pro-429 to Ala-436, Ala-464 to Lys-469, Arg-513 to Asn-520. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution and homology to retroviral envelope proteins indicates that polynucleotides and polypeptides corresponding to this gene would be useful for diagnosis, detection, prevention and/or treatment of cancer and other proliferative disorders, particularly of the endometrium and ovary.

The tissue distribution in infant brain indicates the protein product of this clone would be useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival.

The expression within fetal tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:11 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence

would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2205 of SEQ ID NO:11, b is an integer of 15 to 2219, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:11, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 2

This gene shares sequence homology with members of the B7 family of ligands (i.e., B7-1 (See Genbank Accession 507873)). These proteins and their corresponding receptors play vital roles in the growth, differentiation and death of T cells. For example, some members of this family (i.e., B7-H1) are involved in co-stimulation of the T cell response, as well as inducing increased cytokine production. Therefore, antagonists such as antibodies or small molecules directed against the translation product of this gene are useful for treating T cell mediated immune system disorders.

In additional nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence: LEVQVPEDPVVALVGTDATLCCSFSPEPGFSLAQLNLIWQLTDTKQLVHSFAE GQDQGSAYANRTALFPDLLAQGNASLRLQRVRVADEGSFTCFVSIRDFGSAA VSLQVAAPYSKPSMTLEPNKDLRPGDTVTITCSSYQGYPEAEVFWQDGQGVP LTGNVTTSQMANEQGLFDVHSILRVVLGANGTYSCLVRNPVLQQDAHSSVTI TGQPMTF (SEQ ID NO: 158). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the

invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature extracellular portion of the protein demonstrating functional activity. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Such functional activities include, but are not limited to, biological activity (e.g., T cell costimulatory activity, ability to bind ICOS, and ability to induce or inhibit cytokine production), antigenicity, immunogenicity (ability to generate antibody which binds to a polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention.

Additionally, the translation product of this gene shares sequence homology with butyrophilin and butyrophilin-like molecules (See, e.g., Genbank Accession No. emb|CAB38473.1| (AL034394) dJ1077I5.1 and gb|AAC05288.1| (AF050157); in addition to the following Geneseq Accession Nos. W46488, W97816, W71592, and W78917; all information and references available through these accessions are hereby incorporated herein by reference):

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gb|AAC05288.1| (AF050157) butyrophilin-like [Mus musculus] >sp|O70355|O70355
            BUTYROPHILIN-LIKE (FRAGMENT).
            Length = 452

  Plus Strand HSPs:

 Score = 255 (89.8 bits), Expect = 2.9e-23, Sum P(2) = 2.9e-23
 Identities = 80/292 (27%), Positives = 137/292 (46%), Frame = +1

Query:   613 GPGDMVTITCSSYQGYPEAEVFWQDGQGVPLTGNVTTSQMANEQGLFDVHSILRVVLGAN 792
             G G+ V + C+S   +PE EV W+   G  L  + + + +  E GLF V   L V   +
Sbjct:   156 GEGE-VQLVCTSRGWFPEPEVHWEGIWGEKLM-SFSENHVPGEDGLFYVEDTLMVRNDSV 213

Query:   793 GTYSCLVRNPVLQQDAHSSVTITPQ-RSPTGAVEVQVPEDPVVALVGTDATLHCSFSPEP 969
              T SC + +  L++   +++ ++ + ++   +V V     P    VG +  L C  SP+
Sbjct:   214 ETISCFIYSHGLRETQEATIALSERLQTELASVSVIGHSQPSPVQVGENIELTCHLSPQT 273

Query:   970 GFSLTQLNLIWQLTDTKQLVHSFTEGR----DQGSAYANRTALFPDLLAQGNASLRLQRV 1137
                   L + W  +     VH +  G     +Q   Y  RT+L  D + +G  +L++
Sbjct:   274 --DAQNLEVRWLRSRYYPAVHVYANGTHVAGEQMVEYKGRTSLVTDAIHEGKLTLQIHNA 331

Query:  1138 RVADEGSFTCFVSIRD--FGSAAVSLQVAAPYSKPSMTLEPNKDLRPGDTVTITCSSYRG 1311
             R +DEG + C    +D  +  A V +QV A  S P +T E  KD   G  + + C+S
Sbjct:   332 RTSDEGQYRCLFG-KDGVYQEARVDVQVMAVGSTPRITREVLKD---GG-MQLRCTSDGW 386

Query:  1312 YPEAEVFWQDGQGVPLTGNVTTSQMANEQGLFDVHSVLRVVLGANGTYSCLVRNPVLQQ 1488
             +P   V W+D  G  +       Q  +++ LF V ++L V  G+    +C +  P+ Q+
Sbjct:   387 FPRPHVQWRDRDGKTMPSFSEAFQQGSQE-LFQVETLLLVTNGSMVNVTCSISLPLGQE 444
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 Score = 194 (68.3 bits), Expect = 4.6e-11, P = 4.6e-11
 Identities = 58/210 (27%), Positives = 103/210 (49%), Frame = +1

Query:   901 PEDPVVALVGTDATLHCSFSPEPGFSLTQLNLIWQLTDTKQLVHSFTEGRD-QG---SAY 1068
             P  P++A VG DA L C    P+    +    + + W  +D     V  + +G +  G      Y
Sbjct:    34 PNLPILAKVGEDALLTCQLLPKR--TTAHMEVRWYRSDPDMPVIMYRDGAEVTGLPMEGY 91

Query:  1069 ANRTALFPDLLAQGNASLRLQRVRVADEGSFTCFVSIRDFG-SAAVSLQVAAPYSKPSMT 1245
               R     D    +G+ +L++++V+ +D+G + C      D+    +V LQVAA  S P++
Sbjct:    92 GGRAEWMEDSTEEGSVALKIRQVQPSDDGQYWCRFQEGDYWRETSVLLQVAALGSSPNIH 151

Query:  1246 LEPNKDLRPGDTVTITCSSYRGYPEAEVFWQDGQGVPLTGNVTTSQMANEQGLFDVHSVL 1425
             +E      L  G+ V + C+S     +PE EV W+    G  L  + + + +  E GLF V    L
Sbjct:   152 VE---GLGEGE-VQLVCTSRGWFPEPEVHWEGIWGEKLM-SFSENHVPGEDGLFYVEDTL 206

Query:  1426 RVVLGANGTYSCLVRNPVLQQDAHGSVTITGQPMT 1530
              V    +  T SC + +  L++     ++ ++ +  T
Sbjct:   207 MVRNDSVETISCFIYSHGLRETQEATIALSERLQT 241


 Score = 105 (37.0 bits), Expect = 0.24, P = 0.21
 Identities = 30/100 (30%), Positives = 44/100 (44%), Frame = +2

Query:   254 PVVALVGTDATLCCSFSPEPGFSLAQLNLIWQLTDTKQLVHSFAEGQ----DQGSAYANR 421
             P     VG +  L C  SP+         L + W  +       VH +A G       +Q  Y  R
Sbjct:   254 PSPVQVGENIELTCHLSPQT--DAQNLEVRWLRSRYYPAVHVYANGTHVAGEQMVEYKGR 311

Query:   422 TALFLDLLAQGNASLRLQSVRVADEGQLHLLREHPGFRQRCR 547
             T+L  D + +G  +L++ + R +DEGQ   L    G  Q  R
Sbjct:   312 TSLVTDAIHEGKLTLQIHNARTSDEGQYRCLFGKDGVYQEAR 353


 Score = 97 (34.1 bits), Expect = 2.9e-23, Sum P(2) = 2.9e-23
 Identities = 25/88 (28%), Positives = 44/88 (50%), Frame = +2

Query:   245 PEDPVVALVGTDATLCCSFSPEPGFSLAQLNLIWQLTDTKQLVHSFAEGQD-QG---SAY 412
             P  P++A VG DA L C    P+    + A + + W  +D     V  + +G +  G      Y
Sbjct:    34 PNLPILAKVGEDALLTCQLLPKR--TTAHMEVRWYRSDPDMPVIMYRDGAEVTGLPMEGY 91

Query:   413 ANRTALFLDLLAQGNASLRLQSVRVADEGQ 502
               R     D    +G+ +L+++ V+ +D+GQ
Sbjct:    92 GGRAEWMEDSTEEGSVALKIRQVQPSDDGQ 121
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Butyrophilin is thought to be important in the process of lactation and milk secretion. Based on the sequence similarity, the translation product of this clone is expected to share at least some biological activities with butyrophilin and/or oligodendrite proteins. Such activities are known in the art, some of which are described elsewhere herein.

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence:

ARLGRVPESQSRRGAAGAAFHHGEPSCQPPHRKMLRRRGSPGMGVHVGAAL
GALW
FCLTGALEVQVPEDPVVALVGTDATLCCSFSPEPGFSLAQLNLIWQLTDTKQL

VHSFAEGQDQGSAYANRTALFLDLLAQGNASLRLQSVRVADEGQLHLLREH PGFRQRCRQPAGGRSLLEAQHDPGAQQGPAARGTW (SEQ ID NO: 155). Polynucleotides encoding these polypeptides are also encompassed by the invention.

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence: PWSPTRTCGPGDMVTITCSSYQGYPEAEVFWQDGQGVPLTGNVTTSQMANE QGLFDVHSILRVVLGANGTYSCLVRNPVLQQDAHSSVTITPQRSPTGAVEVQ VPEDPVVALVGTDATLHCSFSPEPGFSLTQLNLIWQLTDTKQLVHSFTEGRDQ GSAYANRTALFPDLLAQGNASLRLQRVRVADEGSFTCFVSIRDFGSAAVSLQ VAAPYSKPSMTLEPNKDLRPGDTVTITCSSYRGYPEAEVFWQDGQGVPLTGN VTTSQMANEQGLFDVHSVLRVVLGANGTYSCLVR NPVLQQDAHGSVTITGQPMTFPPEALWVTVGLSVCLIALLVALPFVCWRKIK QSCEEENAGAEDQDGEGE GSKTALQPLKHSDSKEDDGQEIA (SEQ ID NO: 156). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 15. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 15.

This gene is expressed primarily in dendritic cells and to a lesser extent in fetal liver and spleen, normal colon, and normal liver. It is also expressed in various tumors including ovary, glioblastoma, germ cell tumors, pancreatic tumor, and germinal center B-cell cancer.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to cancer and immune disorders including autoimmune diseases and immuno-deficiency

disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 84 as residues: Glu-72 to Gly-77, Arg-115 to Arg-125, His-138 to Pro-146. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The dendritic cell distribution and homology to the butyrophilin family indicates that polynucleotides and polypeptides corresponding to this gene are useful for down-regulation or stimulation of the immune-response. Dendritic cells play a pivotal role in immune surveillance- they are responsible for the capture and processing of antigens from the periphery and subsequent presentation of these antigens to B and T lymphocytes in lymphoid organs. Dendritic cells also produce and secrete numerous immuno-modulatory proteins. The butyrophilin family appears to have a receptor like structure having an extracellular domain, transmembrane domain and intracellular region. The encoded protein may act as a membrane bound receptor to mediate the interaction of dendritic cells with other cells of the immune system. This interaction could be with either soluble factors produced by other immune cells or with membrane proteins present on other immune cells. Such interactions may result in a stimulation or down-regulation of dendritic cell function. Subsequently the immune system may be stimulated to respond against specific antigens, or the response may dampened as is seen in tolerance of self- antigens. The inability to effectively inhibit immune responses to self antigens could result in auto-immune disease. Conversely the inability to stimulate correct responses could result in an immuno-deficiency syndrome and subsequent susceptibility to infectious agents.

Additionally, the expression of this gene in numerous tumors may reflect the role that this molecule plays in the body's normal anti-tumor surveillance system; tumor cells may express this protein in order to stimulate an immune response (e.g.; targeting of cytotoxic T-cells against the tumor cells). Alternately, the molecule may be used by tumors to dampen the cytotoxic immune response and thus be a means by which tumors escape killing.

Moreover, the tissue distribution in fetal liver spleen and germinal center B-cell indicates the protein product of this clone is useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional

supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:12 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3422 of SEQ ID NO:12, b is an integer of 15 to 3436, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:12, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 3

The translation product of this gene shares sequence homology with matrilin and other cartilage matrix proteins (see, e.g, Genbank Accession Nos. emb|CAA06889.1| (AJ006140); and/or emb|CAA30915.1|; all references available through these accessions are hereby incorporated in their entirety by reference herein). Matrilins are members of a superfamily with von Willebrand factor type A-like modules, which is thought to be important in forming an extracellular, filamentous network.

Moreover, the translation product of this gene also shares sequence homology with the kidney injury associated molecule (KIM) protein (See Geneseq Accession No. W86326; all references and information available through this accession are hereby incorporated herein by reference). Based on the sequence similarity, the translation product of this clone is expected to share at least some biological activities with matrilin, cartilage matrix proteins and KIM proteins. Such activities are known in the art, some of which are described elsewhere herein.

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, an amino acid sequence selected from the group:

KXPCXYRSGIPGSTHASVPSAPRPSRAMLPWTAXGLALSLRLALARSGAERG PPASAPRGDLMFLLDSSASVSHYEFSRVREFVGQLVAPLPLGTGALRASLVHV GSRPYTEFPFGQHSSGEAAQDAVRASAQRMGDTHTGLALVYAKEQLFAEAS GARPGVPKVLVWVTDGGSSDPVGPPMQELKDLGVTVFIVSTGRGNFLELSAA ASAPAEKHLHFVDVDDLHIIVQELRGSILDAMRP (SEQ ID NO: 159); APAWGGPQGRWSRHLSPTPALWAPLAGHLMLQQTAVPWHRPAPGQCGCHP CAGQKHAPHPGQPHPSCAGRRGTRCMADCPRAPDWHAGPRCPGAVEPPAAP QTPEPGRTRSERRWLSCPAGTSGPLGGLMLVDRAPRRSAPAPAASSGPGRXPS RGASRARDGARSARTRGSTREFRTGXCRVXSX (SEQ ID NO: 160), HASVPSAPRPSRAMLPWTALGLALSLRLALARSGAERGPPASAPRGDLMFLL DSSASVSHYEFSRVREFVGQLVAPLPLGTGALRASLVHVGSRPYTEFPFGQHS SGEAAQDAVRASAQRMGDTHTGLALVYAKEQLFAEASGARPGVPKVLVWV TDGGSSDPVGPPMQELKDLGVTVFIVSTGRGNFLELSAAASAPAEKHLHFVD VDDLHIIVQELRGSILDAM (SEQ ID NO: 165); FLLDSSASVSHYEFSRVR (SEQ ID NO: 161), GALRASLVHVGSRP (SEQ ID NO: 162), GVPKVLVWVTDG (SEQ ID NO: 163), and VGPPMQELKDLGVT (SEQ ID NO: 164). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to these polypeptides, or polypeptides encoded by a polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention and polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in uterus, brain, lung, colon, kidney, placenta, dendritic cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: renal, neural, endothelial, developmental, and reproductive diseases and/or disorders, particularly disorders resulting from tissue structural damages or abnormalities, Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell

type(s). For a number of disorders of the above tissues or cells, particularly of the uterus, placenta, kidney, lung, brain, and colon, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., renal, neural, endothelial, developmental, reproductive, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution kidney, combined with the homology to the matrilin and KIM proteins indicates that polynucleotides and polypeptides corresponding to this gene would be useful for treatment, prevention, detection and/or diagnosis of disorders involving tissues with structural damages or abnormalities, particularly organs or tissues such as uterus, placenta, kidney, lung, brain, and colon. Matrilin may be also involved in extracellular transport, storage, barrier of molecular factors such as growth factors, hormones, thereby modulating the organ functions. Representative uses are described in the "Biological Activity", "Hyperproliferative Disorders", "Infectious Disease", and "Regeneration" sections below, in Example 11, 19, and 20, and elsewhere herein.

In addition expression in the placenta indicates that polynucleotides and/or polypeptides corresponding to this gene would be useful in treating, preventing, detecting and/or diagnosing placental related function or diseases, e.g. induced abortion or spontaneous abortion; hyperplastic abnormalities; factors involved in circulation, nutrient transport; prevention of multiple gestation; gestational trophoblastic diseases, such as hydatidiform mole as well as placental site trophoblastic tumor and chriocarcinoma; uterus related function, e.g., disorders during the menstrual cycle or pregnancy, inflammatory changes, such as pyometra, endometritis and dysfunctional bleeding; contraceptives, abortion and birth control; infertility caused by blastocyst, embryo or fetus implantation problems; utilities in surrogate pregnancy; tumors or hyperplasia of the uterus, with epithelium, stroma or smooth muscle origins; brain related functions, e.g., trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, toxic neuropathies induced by neurotoxins, inflammatory diseases such

as meningitis and encephalitis, demyelinating diseases, neurodegenerative diseases such as Parkinson's disease, Huntington's disease, Alzheimer's disease, peripheral neuropathies, multiple sclerosis, neoplasia of neuroectodermal origin, etc; as well as diseases implicated in lung, colon functions. Polynucleotides and/or polypeptides of the invention can be used to promote growth and/or survival of damaged tissue (e.g., renal tissue), since KIM proteins are upregulated in injured or regenerating (especially renal) tissues. Fusion proteins of the invention, conjugates, antibodies and vectors can also be used therapeutically, e.g., these or KIM proteins (or a protein having KIM activity) may be included with an acceptable carrier in pharmaceutical compositions, useful for therapy/prophylaxis of conditions associated with dysfunction/dysregulation of genes or proteins of the invention, especially renal diseases or impairments of renal function in humans (e.g., acute renal failure, acute nephritis). The polynucleotides can be used to produce antisense sequences which, when internalized into cells, can disrupt expression of a cellular gene, also useful in therapy (e.g., to block the growth of tumors dependent on polynucleotides or polypeptides of the invention for growth) or compositions. The proteins and polynucleotides would be useful diagnostically e.g., to detect and quantify renal injury/disease (indicative of increased risk, or presence of, renal injury or impaired function), or abnormal responses to tissue injury (indicative of increased risk, or presence of, an autoimmune response or abnormal tissue growth arising from/affecting renal tissue). The proteins can also be used to locate cells producing the invention (especially specific loci, e.g., tissue masses abnormally producing/expressing polynucleotide or polypeptides of the invention such as tumors arising from/affecting renal tissue), by contacting cells with an imaginable reagent which binds to polynucleotides or polypeptides of the invention and imaging reagent accumulation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:13 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 720 of SEQ ID NO:13, b is an integer of 15 to 734, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:13, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 4

The translation product of this gene shares sequence homology with Liv-1 which is thought to be an estrogen-regulated gene associated with breast cancer. The polypeptide of this gene has been determined to have seven transmembrane domains at about amino acid positions 3-19, 400-436, 433-457, 493-512, 736-753, 758-781, and/or 800-827 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type IIIa membrane proteins.

Included in this invention as preferred domains are zinc finger, C2H2 type, and cytochrome c family heme-binding site signature domains, which were identified using the ProSite analysis tool (Copyright, Swiss Institute of Bioinformatics). 'Zinc finger' domains [1-5] are nucleic acid-binding protein structures first identified in the Xenopus transcription factor TFIIIA. These domains have since been found in numerous nucleic acid-binding proteins.

A zinc finger domain is composed of 25 to 30 amino-acid residues. There are two cysteine or histidine residues at both extremities of the domain, which are involved in the tetrahedral coordination of a zinc atom. It has been proposed that such a domain interacts with about five nucleotides.

A schematic representation of a zinc finger domain is shown below:

x x x x x x x x x x x x C H x / x x Zn x x / x C H x x x x x x x x x x

Many classes of zinc fingers are characterized according to the number and positions of the histidine and cysteine residues involved in the zinc atom coordination. In the first class to be characterized, called C2H2, the first pair of zinc coordinating residues are cysteines, while the second pair are histidines. A number of experimental reports have demonstrated the zinc- dependent DNA or RNA binding property of some members of this class. Some of the proteins known to include C2H2-type zinc fingers are listed below. We have indicated, between brackets, the number of zinc finger regions found in each of these proteins; a '+' symbol indicates that only partial sequence data is available and that additional finger domains may be present. In addition to the conserved zinc ligand residues it has been shown [6] that a number of other positions are also important for the structural integrity of the C2H2 zinc fingers. The best conserved position is found four residues after the second cysteine; it is generally an aromatic or aliphatic residue. The consensus pattern is as follows: C-x(2,4)-C-x(3)-[LIVMFYWC]-x(8)-H-x(3,5)-H (The two C's and two H's are zinc ligands). The following references are referred to above and are hereby incorporated herein by reference: [1] Klug A., Rhodes D., Trends Biochem. Sci. 12:464-469(1987); [2] Evans R.M., Hollenberg S.M., Cell 52:1-3(1988); [3] Payre F., Vincent A., FEBS Lett. 234:245-250(1988); [4] Miller J., McLachlan A.D., Klug A., EMBO J. 4:1609-1614(1985); [5] Berg J.M. Proc. Natl. Acad. Sci. U.S.A. 85:99-102(1988); and [6] Rosenfeld R., Margalit H., J. Biomol. Struct. Dyn. 11:557-570(1993).

In proteins belonging to cytochrome c family [1], the heme group is covalently attached by thioether bonds to two conserved cysteine residues. The consensus sequence for this site is Cys-X-X-Cys-His and the histidine residue is one of the two axial ligands of the heme iron. This arrangement is shared by all proteins known to belong to cytochrome c family, which presently includes cytochromes c, c', c1 to c6, c550 to c556, cc3/Hmc, cytochrome f and reaction center cytochrome c. The consensus pattern is as follows: C-{CPWHF}-{CPWR}-C-H-{CFYW}.
The following reference is referred to above and is hereby incorporated herein by reference: [1] Mathews F.S., Prog. Biophys. Mol. Biol. 45:1-56(1985).

Preferred polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence: CLICLLTFIFHHCNHCHEEHDH (SEQ ID NO:

166) and LLTFIFHHCNHCHEEHDHGPEA (SEQ ID NO: 167). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Further preferred are polypeptides comprising the zinc finger, C2H2 type, and cytochrome c family heme-binding site signature domains of the sequence referenced in Table for this gene, and at least 5, 10, 15, 20, 25, 30, 50, or 75 additional contiguous amino acid residues of this referenced sequence. The additional contiguous amino acid residues may be N-terminal or C- terminal to the zinc finger, C2H2 type, and cytochrome c family heme-binding site signature domains.

Alternatively, the additional contiguous amino acid residues may be both N-terminal and C-terminal to the zinc finger, C2H2 type, and cytochrome c family heme-binding site signature domains, wherein the total N- and C-terminal contiguous amino acid residues equal the specified number. The above preferred polypeptide domain is characteristic of a signature specific to zinc finger, C2H2 type, and cytochrome c family heme-binding site signature domains containing proteins. Based on the sequence similarity, the translation product of this clone is expected to share at least some biological activities with zinc finger and/or cytochrome proteins. Such activities are known in the art, some of which are described elsewhere herein.

The gene encoding the disclosed cDNA is believed to reside on chromosome 2. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 2.

This gene is expressed primarily in brain and hematopoietic tissues and to a lesser extent in breast and pancreas islet cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to:

cancer, particularly breast, brain, and pancreatic cancers; immune system dysfunction; pancreatic disorders and diabetes. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune, CNS, endocrine, and reproductive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 86 as residues: Cys-22 to Asp-30, Glu-45 to Ser-52, Gln-54 to Lys-61, Arg-70 to Arg-76, Ser-125 to His-134, Asn-136 to Thr-141, Ser-146 to Thr-159, Asp-189 to His-194, Phe-196 to Asp-225, Pro-229 to Asn-243, Phe-251 to Val-272, Pro-283 to Leu-305, Thr-308 to Ala-313, Lys-326 to His-333, Ile-388 to Pro-396, His-483 to Leu-489, Tyr-521 to Trp-530, Lys-533 to Glu-538, Lys-544 to Trp-558, Asp-575 to Glu-581, Leu-585 to Asn-595, His-628 to Lys-638, His-645 to His-652, Gly-786 to Gly-794. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in neural tissues, combined with the homology to Liv-1 indicates that polynucleotides and polypeptides corresponding to this gene are useful for the potential diagnosis and/or treatment of cancer, and particularly, though not limited to, brain cancers.

Expression of Liv-1 has been demonstrated to correlate with the incidence of breast cancer; therefore, expression of this Liv-1 homolog may be diagnostic or causative in the development or progression of similar cancers, notably of the breast, brain, and/or pancreas.

Expression of this gene product in hematopoietic cells and tissues also suggests that it may play a role in the normal function of the immune system. Representative uses are described in the "Regeneration" and "Hyperproliferative

Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:14 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 5316 of SEQ ID NO:14, b is an integer of 15 to 5330, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:14, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 5

The translation product of this gene shares sequence homology with prostatic acid phosphatase which is thought to be important in the preservation and maintenance of gastrointestinal mucosa and the repair of acute and chronic mucosal lesions (e.g. enterocolitis, Zollinger-Ellison syndrome, gastrointestinal ulceration and congenital microvillus atrophy), skin diseases associated with abnormal keratinocyte differentiation (e.g. psoriasis, epithelial cancers such as lung squamous cell carcinoma of the vulva and gliomas), potent effects on cell growth and development, diseases related to growth or survival of nerve cells including Parkinson's disease, Alzheimer's disease, ALS, neuropathies or cancer.

This gene is expressed primarily in infant brain and fetal heart and to a lesser extent in smooth muscle cells and fibroblasts.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: fibrosis; neurodegenerative disorders; myocardial infarction; heart defects; cardiac arrhythmias; mucosal lesions; impaired digestive function; cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the cardiovascular, CNS, endocrine, and digestive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, cardiovascular, developmental, and, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, amniotic fluid, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 87 as residues: Thr-34 to Arg-46, Lys-108 to Glu-113, Asn-121 to Lys-128, Lys-186 to Asp-198, Thr-204 to Leu-211, Phe-225 to His-234, Val-249 to Gln-261, Leu-266 to Tyr-275, Glu-330 to Tyr-341, Arg-359 to Glu-369, Asp-410 to His-417, Phe-434 to Pro-445. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution and homology to prostatic acid phosphatase indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of a variety of clinical disorders. Expression of this gene product in brain suggests a possible role or utility in the treatment of neurodegenerative disorders, such as Alzheimers, ALS, or schizophrenia. Expression of this gene product in fibroblasts and smooth muscle cells suggests a possible involvement in the development or progression of fibrotic disorders. Homology to prostatic acid phosphatase suggests a possible involvement in preservation and maintenance of gastrointestinal mucosa and the repair of acute and chronic mucosal lesions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may

show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:15 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2739 of SEQ ID NO:15, b is an integer of 15 to 2753, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:15, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 6

The translation product of this gene shares sequence homology with leptin receptor gene-related protein (OB-RGRP).

This gene is expressed primarily in ovary tumors and a variety of hematopoietic cells and tissues, including dendritic cells and T cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immune system dysfunction; ovarian cancer; T cell lymphomas; inflammation; susceptibility to infection. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and/or reproductive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene

expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 88 as residues: Ala-88 to Gln-98. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in hematopoietic cells and tissues, combined with the homology to a leptin receptor gene-related protein (OB-RGRP) indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of a variety of disorders, including hematopoietic and immune diseases and/or disorders. Homology to leptin receptor gene-related protein (OB-RGRP) suggests that it may play a role in functions mediated by leptin, such as normal appetite. Elevated expression of this gene product in hematopoietic cells and tissues suggests a possible role in normal hematopoiesis, and in the control of the proliferation, survival, activation, and differentiation of blood cell lineages.

Notably, expression on T cells suggests a possible involvement in antigen recognition and the mounting of normal immune responses. Expression on ovarian cancer suggests a possible diagnostic or causative role in the development or progression of this cancer. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host

diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:16 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1339 of SEQ ID NO:16, b is an integer of 15 to 1353, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:16, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 7

The translation product of this gene shares sequence homology with injury-associated molecule, KIM (see, e.g., GeneSeq Accession No. W86309; all references available through this accession are hereby incorporated in their entirety by reference herein) which is thought to be important in promoting tissue growth and regeneration.

The polypeptide of this gene has been determined to have transmembrane domains at about amino acid positions 78 to about 94 and at about 7 to about 23 of the

amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type IIIa membrane proteins.

When tested against human T cells, supernatants removed from cells expressing this gene induced expression of the secreted cytokine, IL-10. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines, e.g.TNF-alpha, IL-1, IL-10, IL-12. Thus, it is likely that the product of this gene is involved in the activation of T cells, in addition to other immune cell-lines or immune tissue cell types. Accordingly, polynucleotides and polypeptides related to this gene may have uses which include, but are not limited to, activating immune cells, such as during an inflammatory response.

This gene is expressed primarily in umbilical vein endothelial cells and to a lesser extent in hepatocellular tumors, breast cancer and bone marrow.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immune disorders, breast cancer and tissue necrosis. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the cardiovascular system, and/or immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 89 as residues: Phe-63 to Phe-70, Arg-107 to Thr-114. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution, homology to injury-associated molecule, and induction of the IL-10 secretion indicates that polynucleotides and polypeptides corresponding to this gene would be useful for tissue / blood vessel regeneration.

Expression in bone marrow indicates that polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis, detection, prevention and/or treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of polynucleotides and polypeptides corresponding to this gene indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. Polynucleotides and polypeptides corresponding to this gene may be involved in the regulation of cytokine production, antigen presentation, or other processes indicating a usefulness in the treatment, detection and/or prevention of cancer (e.g., by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, polynucleotides and polypeptides corresponding to this gene may be involved in immune functions. Therefore it would also be useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, polynucleotides and polypeptides corresponding to this gene are thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

The secreted protein can also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents

that modulate their interactions, and as nutritional supplements. It may also have a very wide range of biological activities. Representative uses are described in the "Chemotaxis" and "Binding Activity" sections below, in Examples 11, 12, 13, 14, 15, 16, 18, 19, and 20, and elsewhere herein. Briefly, polynucleotides and polypeptides corresponding to this gene may possess the following activities: cytokine, cell proliferation/differentiation modulating activity or induction of other cytokines; immunostimulating/immunosuppressant activities (e.g. for treating human immunodeficiency virus infection, cancer (particularly of the breast), autoimmune diseases and allergy); regulation of hematopoiesis (e.g. for treating anemia or as adjunct to chemotherapy); stimulation or growth of bone, cartilage, tendons, ligaments and/or nerves (e.g. for treating wounds, stimulation of follicle stimulating hormone (for control of fertility); chemotactic and chemokinetic activities (e.g. for treating infections, tumors); hemostatic or thrombolytic activity (e.g. for treating hemophilia, cardiac infarction etc.); anti-inflammatory activity (e.g. for treating septic shock, Crohn's disease); as antimicrobials; for treating psoriasis or other hyperproliferative diseases; for regulation of metabolism, and behavior. Also contemplated is the use of the corresponding nucleic acid in gene therapy procedures. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:17 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1024 of SEQ ID NO:17, b is an integer of 15 to 1038, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:17, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 8

This gene is expressed primarily in macrophage and dendritic cells and to a lesser extent in neutrophils.

Polynucleotides and polypeptides of the invention would be useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immune disorders, such as, asthma, arthritis, and chronic inflammatory conditions. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 90 as residues: Pro-55 to His-61. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in macrophage, dendritic cells, and neutrophils indicates that polynucleotides and/or polypeptides corresponding to this gene would be useful for the diagnosis, detection, prevention and/or treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or

other processes suggesting a usefulness in the treatment of cancer (e.g., by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:18 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 704 of SEQ ID NO:18, b is an integer of 15 to 718, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:18, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 9

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence: YXKVRLQVPVRNSRVDPRVRAEVLRATRGGAARGNAAPGRALEMVPGAAG WCCLVLWLPACVAAHGFRIHDYLYFQVLSPGDIRYIFTATPAKDFGGIFHTRY EQIHLVPAEPPEACGELSNGFFIQDQIALVERGGCSFLSKTRVVQEHGGRAVII SDNAVDNDSFYVEMIQDSTQRTADIPALFLLGRDGYMIRRSLEQHGLPWAIIS IPVNVTSIPTFELLQPPWTFW (SEQ ID NO: 168). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to these polypeptides, or polypeptides encoded by a polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention and polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 2. Accordingly, polynucleotides related to this invention would be useful as a marker in linkage analysis for chromosome 2.

Contact of human T cells with supernatant expressing the product of this gene was shown to increase the expression of cell surface molecules, specifically, CD69, CD71 and CD152. Thus it is likely that the product of this gene is involved in the activation of T cells, in addition to other cell-lines or tissue cell types. Therefor, polynucleotides and polypeptides related to this gene have uses which include, but are not limited to, activating immune cells, particularly T cells, such as during an inflammatory response.

This gene is expressed primarily in ovary tumor, and fetal kidney and to a lesser extent in fetal tissues like heart, kidney, liver, bone and broad range distribution in many tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: developmental, reproductive, and renal diseases and/or disorders, particularly

disorders of the ovary or kidney. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the female reproductive system or urinary system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., developmental, reproductive, renal, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 91 as residues: Asp-131 to Ala-137. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in ovarian tissue and activity in cell surface marker assays indicates that polynucleotides and polypeptides corresponding to this gene would be useful for diagnosis, detection, prevention and/or treatment of reproductive disorders, particularly ovary related disease, such as ovarian cancer, as well as cancers of other tissues where expression has been indicated. The expression in ovarian cancer tissue may indicate the gene or its products can be used to treat, prevent, detect and/or diagnose disorders of the ovary, including inflammatory disorders, such as oophoritis (e.g., caused by viral or bacterial infection), ovarian cysts, amenorrhea, infertility, hirsutism, and ovarian cancer (including, but not limited to, primary and secondary cancerous growth, endometrioid carcinoma of the ovary, ovarian papillary serous adenocarcinoma, ovarian mucinous adenocarcinoma, Ovarian Krukenberg tumor). In addition, polynucleotides and polypeptides corresponding to this gene would be useful as a hormone or endocrine factor with either systemic or reproductive functions; growth factors for germ cell maintenance and in vitro culture; fertility control; sexual dysfunction or sex development disorders; Ovarian tumors, such as serous adenocarcinoma, dysgerminoma, embryonal carcinoma, choriocarcinoma, teratoma, etc. Representative uses are described here and elsewhere herein.

The protein product of this clone could be used in the treatment and/or detection of kidney diseases including renal failure, nephritus, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilm's Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:19 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1184 of SEQ ID NO:19, b is an integer of 15 to 1198, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:19, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 10

The polypeptide of this gene has been determined to have three transmembrane domains at about amino acid position 1 to about 27, at about amino acid position 74 to about 93, and at about amino acid position 103 to about 126 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type IIIb membrane proteins.

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, an amino acid sequence selected from the group: HELKMDAEYSGNEFPRSEGERDQHQRPGKERKSGEAGRGTGELGQDGRLLS STLSLSSNRSLGQRQNSPLPFQWRITHSFRWMAQVLASELSLVAFILLLVMAF SKKWLDLSRSLFYQRWPVDVSNRIHTSAHVMSMGLLHFCKSRSCSDLENGK VTFIFSTLMLFPINIWIFELERNVSIPIGWSYFIGWLVLILYFTCAILCYFNHKSF WSLILSHPSGAVSXSSSFGSVEESPRAQTITDTPITQEGVLDPEQKDTHV (SEQ ID NO: 169) and GTSSRWMQSTLGMSSPGQKEKETNIRDLERKGRVGRQDGAQVSWDKMGDC CPPPSPSVVTGPWASARTLRCPFNGESHTASAGWPRCWPLSSAWLPLSYYWS WPSPRNGWTSLGASSTSAGPWMSATESTHQPTLCPWGSCTFANPGAVLT (SEQ ID NO: 170). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to these polypeptides, or polypeptides encoded by a polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention and polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in the testes.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: reproductive diseases and/or disorders, particularly testicular tumors. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, testis, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, seminal fluid, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e.,

the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 92 as residues: Lys-62 to Lys-73. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution primarily in testis indicates that polynucleotides and polypeptides corresponding to this gene would be useful for diagnosis, detection, prevention and/or treatment of cancers of the testis. Polynucleotides and polypeptides corresponding to this gene would be useful for the treatment and diagnosis of conditions concerning proper testicular function (e.g. endocrine function, sperm maturation), as well as cancer. Therefore, this gene product is useful in the treatment of male infertility and/or impotence. This gene product is also useful in assays designed to identify binding agents, as such agents (antagonists) which would be useful as male contraceptive agents. Similarly, the protein is believed to be useful in the treatment and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that is expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications.

In addition, the predicted membrane localization indicates that polynucleotides and/or polypeptides corresponding to this gene would be a good target for antagonists, particularly small molecules or antibodies, which block functional activity (such as, for example, binding of the receptor by its cognate ligand(s); transport function; signaling function). Accordingly, preferred are antibodies and or small molecules which specifically bind an extracellular portion of the translation product of this gene. The extracellular regions can be ascertained from the information regarding the transmembrane domains as set out above. Also provided is a kit for detecting testicular cancer. Such a kit comprises in one embodiment an antibody specific for the translation product of this gene bound to a solid support. Also provided is a method of detecting testicular cancer in an individual

which comprises a step of contacting an antibody specific for the translation product of this gene to a bodily fluid from the individual, preferably serum, and ascertaining whether antibody binds to an antigen found in the bodily fluid. Preferably the antibody is bound to a solid support and the bodily fluid is serum. The above embodiments, as well as other treatments and diagnostic tests (kits and methods), are more particularly described elsewhere herein. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:20 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1019 of SEQ ID NO:20, b is an integer of 15 to 1033, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:20, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 11

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:
ARAEVILCTKEVSVGARKNAFALLVEMGHAFLRFGSNQEEALQCYLVLIYPG
LVGAVTMVSCSILALTHLLFEFKGLMGTSTVEQLLENVCLLLASRTRDVVKS
ALGFIKVAVTVMDVAHLAKHVQLVMEAIGKLSDDMRRHFRMKLRNLFTKFI
RKFGFELVKRLLPEEYHRVLVNIRKAEARAKRHRALSQAAVEEEEEEEEEEP
AQGKGDSIEEILADSEDEEDNEEEERSRGKEQRKLARQRSRAWLKEGGGDEP

LNFLDPKVAQRVLATQPGPAGQEEGPQLQGERRWPADHKGGGRRQQDGGR GRCQRRR (SEQ ID NO: 171). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in immune cells (e.g., B-cells and T-cells), haemopoietic cells and cancer cells (e.g., ovary tumor).

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immune and haemopoietic disorders and cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and haemopoietic system , expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 93 as residues: Leu-77 to Arg-82, Glu-139 to Ser-157, Ser-165 to Arg-191, Glu-196 to Pro-202, Pro-219 to Arg-235, Ala-238 to Arg-259. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in immune cells indicates the protein product of this clone is useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious

Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:21 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

general formula of a-b, where a is any integer between 1 to 1718 of SEQ ID NO:21, b is an integer of 15 to 1732, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:21, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 12

This gene is expressed primarily in germinal B-cells, colon tumor, testes, and anaplastic oligodendrolioma cells and to a lesser extent in a variety of normal and transformed tissues including pooled human melanocyte, fetal heart and pregnant, activated moncytes, chronic lymphotic leukemia.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: cancer and other proliferative disorders, especially colon tumor, immune disorders, and anaplastic oligodendrolioma. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the colon, brain and immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 94 as residues: Leu-53 to Lys-64, Ile-122 to Trp-128, His-149 to Arg-161, Leu-183 to Leu-195. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The expression within fetal tissue and other cellular sources marked by proliferating cells indicates that polynucleotides and/or polypeptides corresponding to this gene may play a role in the regulation of cellular division, and may show utility

in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, including but not limited to colon cancer, prostate cancer, testicular cancer and/or cancer of immune cells), and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention would be useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the tissue distribution in immune cells indicates that polynucleotides and/or polypeptides corresponding to this gene would be useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product in immune cells indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. Polynucleotides and/or polypeptides of the invention may be involved in the regulation of cytokine production, antigen presentation, or other processes indicating that it may be useful in the treatment, and/or prevention of cancer (e.g., by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the

natural gene product would be involved in immune functions. Therefore polynucleotides and/or polypeptides of the invention would also be useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, polynucleotides and/or polypeptides of the invention may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, polynucleotides and/or polypeptides of the invention would be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:22 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 826 of SEQ ID NO:22, b is an integer of 15 to 840, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:22, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 13

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 53 to about 69 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing about amino acids 70 to about 138 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ia membrane proteins.

This gene is expressed primarily in fetal tissue, placenta and breast cancer lymph nodes.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: developmental disorders and breast cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the human fetus, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 95 as residues: Pro-36 to Ala-44, Ile-72 to Trp-77, Gln-94 to Gln-100. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The expression within fetal tissue and other cellular sources marked by proliferating cells indicates that polynucleotides and/or polypeptides corresponding to this gene may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are

described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, polynucleotides and/or polypeptides of the invention may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention would be useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus polynucleotides and/or polypeptides corresponding to this gene may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. Polynucleotides and/or polypeptides of the invention would be useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. The tissue distribution in placenta indicates that polynucleotides and/or polypeptides corresponding to this gene would be useful for the diagnosis and/or treatment of disorders of the placenta. Specific expression within the placenta indicates that polynucleotides and/or polypeptides of the invention may play a role in the proper establishment and maintenance of placental function. Alternately, polynucleotides and/or polypeptides of the invention may be produced by the placenta and then transported to the embryo, where it may play a crucial role in the development and/or survival of the developing embryo or fetus. Expression of this gene product in a vascular-rich tissue such as the placenta also indicates that polynucleotides and/or polypeptides corresponding to this gene may be produced more generally in endothelial cells or within the circulation. In such instances, it may play more generalized roles in vascular function, such as in angiogenesis. It may also be produced in the vasculature and have effects on other cells within the circulation, such as hematopoietic cells. It may serve to promote the

proliferation, survival, activation, and/or differentiation of hematopoietic cells, as well as other cells throughout the body. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:23 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 926 of SEQ ID NO:23, b is an integer of 15 to 940, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:23, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 14

When tested against K592 cell lines, supernatants removed from cells containing this gene activated the ISRE (interferon-sensitive responsive element) promoter element. Thus, it is likely that this gene activates leukemia cells, and to a lesser extent other cells and tissue cell types, through the JAK-STAT signal transduction pathway. ISRE is a promoter element found upstream in many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the ISRE

element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

When tested against HUVEC cells, supernatants removed from cells containing this gene induced phosphorylation of ATF-2. The phosphorylation of ATF-2 occurs as a result of the signaling cascade induced during cell proliferation, thus the phosphorylation state of ATF-2 can be used as a measure of cell proliferation.

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence:
GTREGEGRKCPWKGLRARTGMGQEVHGSCWALGAGGGQRQWVGRSMPPL
APQLCRAVFLVPILLLLQVKPLNGSPGPKDGSQTEKTPSADQNQEQFEEHFVA
SSVGEMWQVVDMAQQEEDQSSKTAAVHKHSFHLSFCFS
LASVMVFSGGPLRRTFPNIQLCFMLTH (SEQ ID NO: 172). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to these polypeptides, or polypeptides encoded by a polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention and polynucleotides encoding these polypeptides are also encompassed by the invention.

The polypeptide encoded by this gene has been determined to have a transmembrane domain at about amino acid position 32 to about 48 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing about amino acids 1 to about 31 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type II membrane proteins.

This gene is expressed primarily in the testes.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: reproductive diseases and/or disorders, particularly testis tumors. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing

immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, testicular, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, seminal fluid, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 96 as residues: Leu-26 to Glu-52, Gln-71 to Lys-79. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in testis, combined with the detected ISRE and ATF-2 biological activity, indicates that polynucleotides and polypeptides corresponding to this gene would be useful for diagnosis, detection, prevention and/or treatment of reproductive system disorders, including cancers of the testis. Polynucleotides and polypeptides corresponding to this gene would be useful for the treatment, prevention, detection and/or diagnosis of conditions concerning proper testicular function (e.g. endocrine function, sperm maturation), as well as cancer. Therefore, this gene product is useful in the treatment of male infertility and/or impotence. Polynucleotides and/or polypeptides of the invention would also be useful in assays designed to identify binding agents, as such agents (antagonists) are useful as male contraceptive agents. Similarly, polynucleotides and/or polypeptides of the invention are believed to be useful in the treatment, prevention, detection and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that is expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications. In addition, the predicted membrane localization indicates that polynucleotides and/or polypeptides corresponding to this gene would be a good target for antagonists,

particularly small molecules or antibodies, which block functional activity (such as, for example, binding of the receptor by its cognate ligand(s); transport function; signaling function). Accordingly, preferred are antibodies and or small molecules which specifically bind an extracellular portion of the translation product of this gene. The extracellular regions can be ascertained from the information regarding the transmembrane domains as set out above. Also provided is a kit for detecting testicular cancer. Such a kit comprises in one embodiment an antibody specific for the translation product of this gene bound to a solid support. Also provided is a method of detecting testicular cancer in an individual which comprises a step of contacting an antibody specific for the translation product of this gene to a bodily fluid from the individual, preferably serum, and ascertaining whether antibody binds to an antigen found in the bodily fluid. Preferably the antibody is bound to a solid support and the bodily fluid is serum. The above embodiments, as well as other treatments and diagnostic tests (kits and methods), are more particularly described elsewhere herein. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:24 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 787 of SEQ ID NO:24, b is an integer of 15 to 801, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:24, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 15

The translation product of this gene shares sequence homology with EMILIN (see, e.g., Genbank Accession No. gb|AAD42161.1|AF088916_1 (AF088916); all references available through this accession are hereby incorporated in their entirety by reference herein). EMILIN (elastin microfibril interface located protein), an extracellular matrix glycoprotein, is thought to be important in cell adhesion and cell-to-cell communication, especially in elastic tissues.

This gene is expressed in pregnant uterus, uterine cancer, breast cancer, pancreatic cancer, fetal kidney, whole embryo, and to a lesser extent, in human thymus and colon.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: circulatory, growth and developmental defects, including, but not limited to cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the cardiovascular and musculoskeletal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, developmental, gastrointestinal, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, amniotic fluid, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 97 as residues: Phe-30 to Cys-37, Arg-91 to Gly-98, Pro-170 to Ala-177, Pro-183 to Gly-193, Pro-206 to Gly-235, Pro-243 to Pro-260, Phe-283 to Gly-311. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in uterus, combined with the homology to EMILIN indicates that polynucleotides and polypeptides corresponding to this gene would be useful for study, treatment, prevention, detection and/or diagnosis of disorders of

growth and development, blood vessel and other elastic tissue integrity and function, and fibrotic and neoplastic conditions. Polynucleotides and/or polypeptides of the invention would be useful in the detection, treatment, and/or prevention of vascular conditions, which include, but are not limited to, microvascular disease, vascular leak syndrome, aneurysm, stroke, atherosclerosis, arteriosclerosis, or embolism. For example, this gene product may represent a soluble factor produced by smooth muscle that regulates the innervation of organs or regulates the survival of neighboring neurons. Likewise, it may be involved in controlling the digestive process, and such actions as peristalsis. Similarly, it may be involved in controlling the vasculature in areas where smooth muscle surrounds the endothelium of blood vessels. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:25 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1955 of SEQ ID NO:25, b is an integer of 15 to 1969, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:25, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 16

The polypeptide of this gene has been determined to have transmembrane domains at about amino acid positions 9-25, 32-48, and 188-204 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is

believed that the protein product of this gene shares structural features to type IIIb membrane proteins.

Moreover, in specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:MDFIQHLGVCCLVALISVGLLSVAACWFLPSIIAAAASWIITCVLLCC SKHARCFILLVFLSCGLREGRNALIAAGTGIVILGHVENIFHNFKGLLDGMTCN LRAKSFSIHFPLLKKYIEAIQWIYGLATPLSVFDDLVSWNQTLAVSLFSPSHVL EAQLNDSKGEVLSVLYQMATTTEVLSSLGQKLLAFAGLSLVLLGTGLFMKRF LGPCGWKYENIYITRQFVQFDERERHQQRPCVLPLNKEERRKFISGFQS (SEQ ID NO:). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in macrophages, monocytes, dendritic cells, T-cell lymphoma and osteoclastoma.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immunodeficiency, infection, lymphoma, auto-immunity, cancer, inflammation, anemia (leukemia) and other hematopoeitic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene

expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 98 as residues: Asp-229 to Gln-236, Asn-244 to Lys-250, Trp-258 to Asn-266. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in immune cells (e.g., dendritic cells and macrophage) indicates the protein product of this clone is useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional

supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:26 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1350 of SEQ ID NO:26, b is an integer of 15 to 1364, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:26, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 17

The polypeptide of this gene has been determined to have a transmembrane domains at about amino acid positions 10-26, 157-173, and 67-83 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type IIIb membrane proteins.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:
MAGGWAAEAVWAGFGVVVVARRLVLLPLLLHPGFQQLLLVLLLPHEQLHH
EHLLLVDLLADVLGDVRDDPVHKVAHEHDQVLEDDDKRQPGCQDGPEVLG
DVVLVFRPRRLSVVFIPADLHLVAQVQGVIGGRAVLEVTDVEGGEGVVDEA
VHGPVLTVHVEVHQARDEVRREGDHEGIDDDSKLPNASEDIVPDSDVFGSDS
YRPSELSDKLFGVQADLDDVVQQRKQWGQGEGGDKQGDEAKLDDH
FHVLWGEAREGLQVVIHLV (SEQ ID NO: 173). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,

under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in pituitary tissue, fetal heart, B-cell lymphoma, testes, ovarian cancer, prostate, tumors of the endometrium, parathyroid, pancreas, and to a lesser extent in activated T-cells and broad range of tissues at lower levels.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders related to ovary function, endocrinological disorders, cancer of the endometrium, parathyroid, B-cells, colon, and cancer, in general, as well as, cardiovascular diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive system, endocrine system or cardiovascular system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 99 as residues: Asp-113 to Leu-124, Arg-134 to Lys-152, Arg-207 to Leu-215, Glu-221 to Ala-238. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of disorders related to endocrine disorders, such as disorders of growth, somatic and sexual development, reproductive functions, and metabolic regulation, either as the result of hypopituitarism or hyperpituitarism.

The expression in ovary indicates the gene function as hormone with either systemic or reproductive functions; growth factors for germ cell maintenance and in vitro culture; fertility control; sexual dysfunction or sex development disorders; Ovarian tumors, such as serous adenocarcinoma, dysgerminoma, embryonal carcinoma, choriocarcinoma, teratoma, etc; The expression in heart indicates the gene function and uses in heart failure, congenital heart diseases, ischemic heart diseases, rheumatic/hypersensitivity diseases, cardiomyopathy, luetic heart disease, inflammatory diseases of the heart, hypertensive heart disease, nutritional, endocrine, and metabolic diseases of the heart.

The tissue distribution in testes tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of male reproductive and endocrine disorders. It may also prove to be valuable in the diagnosis and treatment of testicular cancer, as well as cancers of other tissues where expression has been observed.

Moreover, the expression within fetal tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of

degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:27 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2357 of SEQ ID NO:27, b is an integer of 15 to 2371, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:27, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 18

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 103 to about 119 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing about amino acids 120 to about 127 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ia membrane proteins.

The gene encoding the disclosed cDNA is believed to reside on chromosome 10. Accordingly, polynucleotides related to this invention would be useful as a marker in linkage analysis for chromosome 10.

This gene is expressed primarily in fetal tissue, ovary tumor, kidney tumor, brain and to a lesser extent in many other tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: developmental, neurological and behavioral disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous and developmental systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 100 as residues: Leu-18 to Ile-28, His-72 to Trp-93. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in brain indicates that polynucleotides and/or polypeptides corresponding to this gene would be useful for the detection, diagnosis, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated

expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, polynucleotides and/or polypeptides of the invention would be involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. The expression within fetal tissue and other cellular sources marked by proliferating cells indicates that polynucleotides and/or polypeptides of the invention may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, polynucleotides and/or polypeptides of the invention may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention would be useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus, polynucleotides and/or polypeptides corresponding to this gene may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. Polynucleotides and/or polypeptides of the invention would be useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as,

antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:28 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 853 of SEQ ID NO:28, b is an integer of 15 to 867, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:28, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 19

The polypeptide of this gene has been determined to have transmembrane domains at about amino acid position 4-20 and 38-54 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type IIIa membrane proteins.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:
PRAAGIRHELIHGLWNLVFLFSNLSLIFLMPFAYFFTESEGFAGSRKGVLGRVY
ETVVMLMLLTLLVLGMVWVASAIVDKNKANRESLYDFWEYYLPYLYSCISF
LGVLLLLGECTGSGREWAGSLDQSNQARRKGNGGHVREGVESRVWQVTGS
CPYSVYSTGSRPHVLRHWEAASQAPAAGRPGGAAVLLSL (SEQ ID NO: 174).
Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the

invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in vascular endothelial cells, immune cells (T-cells, neutrophils, and dendritic cells), small intestine, and tumors such as ovary tumor, and to a lesser extent in a wide variety of human tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immune disorders, cancers such as ovary tumor. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the vascular system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 101 as residues: Asp-21 to Ser-29, Thr-58 to Trp-64, Asp-69 to Gly-81. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of cancers and diseases related to blood vessel abnormality such as ischemia. The tissue distribution in immune cells indicates the protein product of this clone is useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including

blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:29 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1591 of SEQ ID NO:29, b is an integer of 15 to 1605, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:29, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 20

The gene encoding the disclosed cDNA is believed to reside on chromosome 16. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 16.

This gene is expressed primarily in breast, infant brain and 9 week early human, fetal liver spleen, and to a lesser extent in fetal brain.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: neurodevelopmental, reproductive, immune, and hematopoietic diseases and/or disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, reproductive, breast, brain, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, breast milk, amniotic fluid, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 102 as residues: Arg-125 to Gly-130, Lys-138 to Phe-144. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in infant brain indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of neurodevelopmental disorders. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11,

15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Moreover, the expression within fetal tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:30 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1320 of SEQ ID NO:30, b is an integer of 15 to 1334, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:30, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 21

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence:
HASAFFGTRALLSVSLPPPCMLHWVLSFFFLLSCPRTEGLPGLYCPGCSQCPG
RGMWPGDPGPGIQGPGLDLRTGMEATGAQQPTLSSPHCLLSLPTLPARAVQL
RWDLSISRAGGRVAVLGLCLEPGGSLLLPPSALPE
TDPCAACPPCPFVPMSGGGGRPTVPEAGHQP (SEQ ID NO: 175).
Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in ovarian tumor and to a lesser extent in B-cells (stimulated), Primary Breast Cancer, melanocyte, Pituitary, subtracted, Breast Cancer Cell line, angiogenic, 12 Week Old Early Stage Human, Osteoblasts, Soares adult brain N2b5HB55Y, and Hemangiopericytoma.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: ovarian cancer, developmental, reproductive, and immune diseases and/or disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the female reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, skeletal, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, breast milk, amniotic fluid, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 103 as residues:

Ser-29 to Met-36, Gly-60 to Ser-67. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in ovarian cancer tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of ovarian cancer. Moreover, the expression within fetal tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:31 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 997 of SEQ ID NO:31, b is an integer of 15 to 1011, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:31, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 22

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 15 to about 31 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing about amino acids 1 to about 14 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type II membrane proteins.

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, an amino acid sequence selected from the group: SHTRPTEQPSVLPLFMMYVMMAYLTLFQMGSWMSFSLSLCSLLFILTGHCLS ENFYVRGDGTRAYFFTKGEVHSMFCKASLDEKQNLVDRRLQVNRKKQVKM HRVWIQGKFQKPLHQTQNSSNMVSTLLSQD (SEQ ID NO: 176); and ARESSWDHVKTSATNRFSRMHCPTVPDEKNHYEKSSGSSEGQSKTESDFSNL DSEKHKKGPMETGLFPGSNATFRILEVGCGAGNSVFPILNTLENSPESFLYCC DFASGAVELVKSHSSYRATQCFAFVHDVCDDGLPY PFPDGILDVILLVFVLSSIHPDRTLFI (SEQ ID NO: 177). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical

to these polypeptides, or polypeptides encoded by a polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention and polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in bone marrow as well as osteoclastoma, breast, prostate and colon cancers.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diseases and/or disorders of immune cells and tissues, breast, prostate, colon, in addition to leukemia, osteoclastoma and other cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and hematopoeitic systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., breast, prostate, colon, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, breast milk, seminal fluid, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 104 as residues: Phe-35 to Thr-42, Leu-61 to Val-68, Asn-75 to Val-80, Gly-89 to Ser-102. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in bone marrow indicates that polynucleotides and polypeptides corresponding to this gene may be useful in the treatment and diagnosis of cancers and pathologies associated with neoplastic or proliferative states. The expression in bone marrow would suggest a role in hematopoeitic conditions, anemias (leukemias), auto-immunities, immunodeficiencies, immuno-supressive conditions (e.g., transplantation), inflammation and general microbial infection. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below,

in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Polynucleotides and/or polypeptides of the invention would be useful in modulating the immune response to aberrant polypeptides, as may be present in rapidly proliferating cells and tissues, including cancers. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:32 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1294 of SEQ ID NO:32, b is an integer of 15 to 1308, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:32, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 23

This gene is expressed primarily in activated monocytes.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample

and for diagnosis of diseases and conditions which include but are not limited to: immunodeficiency, infection, lymphoma, auto-immunity, cancer, inflammation, anemia (leukemia) and other hematopoeitic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 105 as residues: Gln-36 to Leu-43, Phe-50 to Thr-57. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in activated monocytes indicates the protein product of this clone is useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as

autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:33 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1420 of SEQ ID NO:33, b is an integer of 15 to 1434, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:33, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 24

This gene is expressed primarily in fetal and adult brain, esp. in cortical structures, and to a lesser extent in lung.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: neurological and pulmonary conditions. Similarly, polypeptides and antibodies

directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the CNS and cardiopulmonary systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 106 as residues: Val-40 to Thr-51. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in brain indicates the protein product of this clone is useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies

directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:34 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2170 of SEQ ID NO:34, b is an integer of 15 to 2184, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:34, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 25

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence: HEQEPLPAPVAEAALPSARNSSVLASLSPHTGPAGLLRDSSVQVSTLGCLLGC GGRMFFPCLPTLXLRIL HSGWVGLFLLISSRAPSSSLAWKHGPGELWWPRXPLRSCTGLASCG (SEQ ID NO: 178). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in salivary gland, pancreas tumor and cerebellum.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: neuroendocrine, metabolic conditions and tumors. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the CNS and endocrine system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for study and treatment of general hormonal, metabolic, neuroendocrine and memory disorders and neoplasms. The tissue distribution in brain indicates the protein product of this clone is useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. The tissue distribution in

pancreas suggests that the protein product of this clone is useful for the detection, treatment, and/or prevention of various endocrine disorders and cancers, particularly Addison's disease, Cushing's Syndrome, and disorders and/or cancers of the pancreas (e.g. diabetes mellitus), adrenal cortex, ovaries, pituitary (e.g., hyper-, hypopituitarism), thyroid (e.g. hyper-, hypothyroidism), parathyroid (e.g. hyper-, hypoparathyroidism) , hypothallamus, and testes. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:35 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1282 of SEQ ID NO:35, b is an integer of 15 to 1296, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:35, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 26

This gene is expressed primarily in neutrophils and T-cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above

tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 108 as residues: Ser-22 to His-40. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in immune cells (e.g., neutrophils and T-cells) indicates the protein product of this clone is useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene

product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:36 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1284 of SEQ ID NO:36, b is an integer of 15 to 1298, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:36, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 27

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 28 - 44 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 45 to 97 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ib membrane proteins.

The gene encoding the disclosed cDNA is believed to reside on chromosome 5. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 5.

This gene is expressed primarily in brain and to a lesser extent in skeletal muscle, pregnant uterus.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: neurodegenerative disease states, behavioral disorders and in general disorders of the CNS, and developmental conditions and diseases, skeletal muscle diseases. . Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the CNS, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, amniotic fluid, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in brain indicates that polynucleotides and polypeptides corresponding to this gene are useful for detection, treatment, and/or prevention of a variety of CNS disorders, including neurodegenerative disease states, behavioral disorders. In addition, polynucleotides and polypeptides corresponding to this gene are useful for detection, treatment, and/or prevention of developmental disorders, skeletal muscle diseases. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse

formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:37 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 539 of SEQ ID NO:37, b is an integer of 15 to 553, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:37, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 28

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence: LTPALPSPRSASPLLSPESLQSPQWPSSSLSIHSLPVAGKPSLITSLFTEPCDGFM AIRGSNTQGLTMMTMTSDRWFSMAWASCSLSRPPLTPSCSCQQPATVALLLQ TISVCSAQQADPLSPPRACRPXRQFPVLQSAGPPHSPHVYAFVLFPVSSRWQG GDFCXICCCFPQCLGRCLEHTRCSINPX (SEQ ID NO: 179). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which

hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in breast cancer tissue.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: reproductive diseases and/or disorders, particularly cancer and other hyperproliferative diseases and/or conditions. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive system or secretory/ductile tissues, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, breast, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, breast fluid, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 110 as residues: Gln-49 to Cys-60. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in breast cancer tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for detection, treatment, and/or prevention of breast neoplasia and breast cancers, such as, but not limited to fibroadenoma, papillary carcinoma, ductal carcinoma, Paget's disease, medullary carcinoma, mucinous carcinoma, tubular carcinoma, secretory carcinoma and apocrine carcinoma, as well as juvenile hypertrophy and gynecomastia, mastitis and abscess, duct ectasia, fat necrosis and fibrocystic diseases. Representative uses are

described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:38 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 587 of SEQ ID NO:38, b is an integer of 15 to 601, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:38, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 29

This gene is expressed primarily in IL-1 and LPS induced neutrophils

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immune system disorders and sepsis. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level,

i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 111 as residues: Glu-17 to Lys-30, Val-43 to Asn-53. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in neutrophils indicates that polynucleotides and polypeptides corresponding to this gene would be useful for modulating the response of activated neutrophils and may thus be important for regulating acute allergic responses such as occurs in sepsis. In addition, polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes indicating a usefulness in the treatment of cancer (e.g., by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of

various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:39 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1880 of SEQ ID NO:39, b is an integer of 15 to 1894, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:39, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 30

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence: RLCRETALMSLCLVLMRRMGWIDLLLPELGALRVFLHLFLVALRTKRWIFRT LGQLTCVNILGDSRKKRECRLNKRQLQFGEKTLQVPERLVVRHSPF (SEQ ID NO: 180). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to these polypeptides, or polypeptides encoded by a polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention and polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in spinal cord, retina and prostate.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: retinal dysplasia, retinitis, choroideremia, diabetic retinopathy, retinal degeneration, retinal detachment, prostate disorders, prostate cancer, spinal trauma, meningitis, spina bifida, spinal tumors and neoplasms, as well as other developmental and neurodegenerative conditions of the spinal cord and central nervous system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the retina and nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., skeletal, neural, reproductive, visual, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, aqueous humor, vitreous humor, seminal fluid, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 112 as residues: Gly-45 to Gln-59, Phe-62 to Leu-67. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The expression in retina indicates that polynucleotides and polypeptides corresponding to this gene would be useful for treatment, prevention, detection and/or diagnosis of retinal dysplasia, retinitis, choroideremia, diabetic retinopathy, retinal degeneration and detachment. The expression in prostate indicates that polynucleotides and polypeptides corresponding to this gene would be useful in the treatment, prevention, detection and/or diagnosis of prostate disorders, particularly prostate cancer, as well as cancers of other tissues where expression has been indicated. Expression in prostate tissue indicates the gene or its products would be useful for diagnosis, treatment and/or prevention of the disorders of the prostate, including inflammatory disorders, such as chronic prostatitis, granulomatous prostatitis and malacoplakia, prostatic hyperplasia and prostate neoplastic disorders,

including adenocarcinoma, transitional cell carcinomas, ductal carcinomas, squamous cell carcinomas, or as hormones or factors with systemic or reproductive functions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. In addition, the expression in spinal cord indicates a role for the polynucleotides and polypeptides corresponding to this gene in the treatment, prevention, detection and/or diagnosis of spinal trauma, meningitis, spina bifida, spinal tumors and neoplasms as well as other developmental and neurodegenerative conditions of the spinal cord and central nervous system. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:40 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3265 of SEQ ID NO:40, b is an integer of 15 to 3279, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:40, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 31

This gene is expressed primarily in ovarian tumor.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to:

disorders of the reproductive system, including ovarian cancer and/or other cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, ovarian, and cancerous and wounded tissues) or bodily fluids (e.g., vaginal pool, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in ovarian tumor indicates that polynucleotides and polypeptides corresponding to this gene would be useful for the detection, diagnosis, prevention and/or treatment of developmental anomalies, fetal deficiencies, pre-natal disorders or ovarian and endometrial cancers, as well as cancers of other tissues where expression has been indicated. The expression in ovarian cancer tissue may indicate the gene or its products can be used to treat, prevent, detect and/or diagnose disorders of the ovary, including inflammatory disorders, such as oophoritis (e.g., caused by viral or bacterial infection), ovarian cysts, amenorrhea, infertility, hirsutism, and ovarian cancer (including, but not limited to, primary and secondary cancerous growth, endometrioid carcinoma of the ovary, ovarian papillary serous adenocarcinoma, ovarian mucinous adenocarcinoma, Ovarian Krukenberg tumor). In addition, the expression in this particular form of cancer, may suggest a role in the treatment and diagnosis of other cancers or pathologies associated with neoplastic or proliferative states. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to

control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:41 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3081 of SEQ ID NO:41, b is an integer of 15 to 3095, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:41, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 32

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 18-34 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 1 - 17 of this protein has also been determined. Based upon these characteristics, it is

believed that the protein product of this gene shares structural features to type II membrane proteins.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:
MLLPFIKLPTTGNSLAKIQTVGQNQQKVNRVLMGPRSIQKRHFKEVGRQSIRR EQGAQASVENAAEEKRLGSPAPRELEQPHTQQGPEKLAGNAIYTKPSFTQEH KAAVSVLTPFSKGAPSTSSPAKALPQVRDRWKDNTHTISILESAKARVTNMK ASKPISHSRKKYRFHKTRSRMTHRTPKVKKSPKFRKKSYLSRLMLANRPPFSA AKSLINSPSQGAFSSLGDLSPQENPFLEVSAPSEHFIETTNIKDTTARNALEENV FMENTNMPEVTISENTNYNHPPEADSAGTAFNLGPTVKQTET NSC (SEQ ID NO: 181). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in fetal tissue (e.g., lung, heart), brain, immune cells (e.g., T-cells, B-cell lymphoma) duodenum, ovary tumor, cheek carcinoma, adipose tissue, CD34+ cells and to a lesser extent, ubiquitously expressed in many tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immune disorders, disorders of the CNS, gastrointestinal tract disorders, ovary dysfunctions, or neoplasia. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the CNS, immune system, gastrointestinal and reproductive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and

wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 114 as residues: Glu-35 to Phe-44. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of gastrointestinal disorders, such as gastritis, peptic ulcer disease, neoplasia of duodenal and/or ovarian origins. The tissue distribution in brain indicates the protein product of this clone is useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. The tissue distribution in immune cells (e.g., B-cells, T-cells) indicates the protein product of this clone is useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation;

survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Moreover, the expression within fetal tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the

polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:42 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2306 of SEQ ID NO:42, b is an integer of 15 to 2320, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:42, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 33

This gene is expressed primarily in colon cancer, Gessler Wilms tumor, brain, breast cancer, fetal tissue and to a lesser extent in ovary tumor, adrenal gland and many other tissues at lower levels.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample

and for diagnosis of diseases and conditions which include but are not limited to: disorders of the developing fetus, central nervous system (CNS), colon cancers or tumors of other origins. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the cancers of colon and ovary, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 115 as residues: Lys-60 to Ser-74. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in ovary cancer and colon indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of colon cancer, ovary cancer or other cancer types. The tissue distribution in kidney suggests that this gene or gene product is useful in the treatment and/or detection of kidney diseases including renal failure, nephritus, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilms Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. The expression within fetal tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can

result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation.

The tissue distribution in brain indicates the protein product of this clone is useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions,

in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:43 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2393 of SEQ ID NO:43, b is an integer of 15 to 2407, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:43, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 34

This gene is expressed primarily in osteoclastoma.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: bone disorders, for example osteoclastoma and osteoporosis. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the skeletal system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in osteoclastoma indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of osteoclastoma and osteoporosis. The secreted protein can also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, and as nutritional supplements. It may also have a very wide range of biological activities. Representative uses are described in the "Chemotaxis" and "Binding Activity" sections below, in Examples 11, 12, 13, 14, 15, 16, 18, 19, and 20, and elsewhere herein. Briefly, the protein may possess the following activities: cytokine, cell proliferation/differentiation modulating activity or induction of other cytokines; immunostimulating/immunosuppressant activities (e.g. for treating human immunodeficiency virus infection, cancer, autoimmune diseases and allergy); regulation of hematopoiesis (e.g. for treating anemia or as adjunct to chemotherapy); stimulation or growth of bone, cartilage, tendons, ligaments and/or nerves (e.g. for treating wounds, stimulation of follicle stimulating hormone (for control of fertility); chemotactic and chemokinetic activities (e.g. for treating infections, tumors); hemostatic or thrombolytic activity (e.g. for treating hemophilia, cardiac infarction etc.); anti-inflammatory activity (e.g. for treating septic shock, Crohn's disease); as antimicrobials; for treating psoriasis or other hyperproliferative diseases; for regulation of metabolism, and behavior. Also contemplated is the use of the corresponding nucleic acid in gene therapy procedures. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:44 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1916 of SEQ ID NO:44, b is an integer of 15 to 1930, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:44, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 35

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence: LKEMAELHHGRSTSLCILPLQRTRIHSMSASLWCFRSQQSIPMRC HRSLSEIPEDFQMNRSTRSYRCWATWPRLGWALPCCMNSLRKGRKFSQITTS LMASVSSASMVSRRRRPL PKHPVTTTSTATALLGTSSTWSKS (SEQ ID NO: 182). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence: TRPDWVLPSEVEVLESIYLDELQVIKGNGRTSPWEIYITLHPATAEDQDSQYV CFTLVLQVPAEYPHEVPQISIRNPRGLSDEQIHTILQVLGHVAKAGLGTA (SEQ ID NO: 183) and MLYELIEKGKEILTDNNIPHGQCVICLYGFQEKEAFTKTPCYHYFHCHCLARY IQHMEQELKAQGQEQEQERQHATTKQKAVGVQCPVCREPLVYDLASLKAAP EPQQPMELYQPSAESLRQQEERKRLYQRQQERGGIIDLEAERNRYFISLQQPP APAEPESAVDVSKGSQPPSTLAAELSTSPAVQSTLPPPLPVATQHICEKIPGTRS NQQRLGETQKAMLDPPKPSRGPWRQPERRHPKGGECHAPKGTRDTQELPPPE GPLKEPMDLKPEPHSQGVEGPPQEKGPGSWQGPPPRRTRDCVRWERSKGRTP GSSYPRLPRGQGAYRPGTRRESLGLESKDGS (SEQ ID NO: 184). Moreover,

fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in Pooled human melanocyte, fetal heart, and pregnant and to a lesser extent in Adult Testes, and germinal center B cell.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: integumentary, cardiovascular, and developmental diseases and/or disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the fetal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., integumentary, cardiovascular, and developmental, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 117 as residues: Met-1 to Thr-13, Ser-27 to Phe-34, Arg-53 to Pro-59, Ser-77 to Ser-82. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in human melanocyte indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of developmental disorders. Representative uses are described in the "Biological Activity", "Hyperproliferative Disorders", "Infectious Disease", and "Regeneration"

sections below, in Example 11, 19, and 20, and elsewhere herein. Briefly, the protein is useful in detecting, treating, and/or preventing congenital disorders (i.e. nevi, moles, freckles, Mongolian spots, hemangiomas, port-wine syndrome), integumentary tumors (i.e. keratoses, Bowen's disease, basal cell carcinoma, squamous cell carcinoma, malignant melanoma, Paget's disease, mycosis fungoides, and Kaposi's sarcoma), injuries and inflammation of the skin (i.e.wounds, rashes, prickly heat disorder, psoriasis, dermatitis), atherosclerosis, uticaria, eczema, photosensitivity, autoimmune disorders (i.e. lupus erythematosus, vitiligo, dermatomyositis, morphea, scleroderma, pemphigoid, and pemphigus), keloids, striae, erythema, petechiae, purpura, and xanthelasma. In addition, such disorders may predispose increased susceptibility to viral and bacterial infections of the skin (i.e. cold sores, warts, chickenpox, molluscum contagiosum, herpes zoster, boils, cellulitis, erysipelas, impetigo, tinea, althletes foot, and ringworm).

Moreover, the protein product of this clone may also be useful for the treatment or diagnosis of various connective tissue disorders (i.e., arthritis, trauma, tendonitis, chrondomalacia and inflammation, etc.), autoimmune disorders (i.e., rheumatoid arthritis, lupus, scleroderma, dermatomyositis, etc.), dwarfism, spinal deformation, joint abnormalities, amd chondrodysplasias (i.e. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:45 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention

are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1445 of SEQ ID NO:45, b is an integer of 15 to 1459, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:45, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 36

This gene is expressed primarily in ovarian tumor and to a lesser extent in Adult Pulmonary.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: reproductive diseases and/or disorders, particularly ovarian cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the female reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, pulmonary, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, pulmonary lavage, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 118 as residues: Pro-28 to Ser-35. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in ovarian tumor tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of ovarian cancer. Moreover, the expression within cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular

division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. The protein is useful in the detection, treatment, and/or prevention of pulmonary diseases and/or disorders, which include, but are not limited to ARDS and emphysema. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:46 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence

would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 989 of SEQ ID NO:46, b is an integer of 15 to 1003, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:46, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 37

The translation product of this gene shares sequence homology with vesicle trafficking protein (see, e.g., Genbank Accession number AAD02171.1 (AF039568); all references available through this accession are hereby incorporated by reference herein.) which is thought to be important in the elaborate transport machinery and cell trafficking system. The polypeptide of this gene has been determined to have transmembrane domains at about amino acid positions 114-130 and 150-166 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type IIIa membrane proteins.

This gene is expressed primarily in melanocytes, fetal tissue, placenta, and testes and to a lesser extent in many other tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: fetal development and endocrine disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e.,

the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Homology to vesicle trafficking protein and the expression within fetal tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:47 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1344 of SEQ ID NO:47, b is an integer of 15 to 1358, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:47, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 38

This gene is expressed primarily in Saos2 cell line (Dexamethosome Treated), IL-1/TNF stimulated Synovial Fibroblasts, osteoblasts, pancreas tumor, retina, hepatocellular tumor (re-excision), and 8 Week Whole Embryo.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: cancer and other proliferative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 120 as residues: Pro-8 to Gly-21, Cys-44 to Tyr-52, Thr-60 to Glu-75, Asp-205 to Ala-223, Thr-372 to Arg-385, Gly-468 to Thr-483, Arg-491 to Gln-500, Lys-537 to Asp-543, Asp-573 to

Ser-583, Pro-586 to Ala-593. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The expression of this gene product in synovium indicates that polynucleotides and/or polypeptides corresponding to this gene would be useful in the detection, diagnosis, prevention and/or treatment of disorders and conditions affecting the skeletal system, in particular osteoporosis as well as disorders afflicting connective tissues (e.g. arthritis, trauma, tendonitis, chrondomalacia and inflammation), such as in the diagnosis or treatment of various autoimmune disorders such as rheumatoid arthritis, lupus, scleroderma, and dermatomyositis as well as dwarfism, spinal deformation, and specific joint abnormalities as well as chondrodysplasias (ie. spondyloepiphyseal dysplasia congenita, familial arthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid).

The protein can also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, and as nutritional supplements. It may also have a very wide range of biological activities. Representative uses are described in the "Chemotaxis" and "Binding Activity" sections below, in Examples 11, 12, 13, 14, 15, 16, 18, 19, and 20, and elsewhere herein. Briefly, the protein may possess the following activities: cytokine, cell proliferation/differentiation modulating activity or induction of other cytokines; immunostimulating/immunosuppressant activities (e.g. for treating human immunodeficiency virus infection, cancer, autoimmune diseases and allergy); regulation of hematopoiesis (e.g. for treating anemia or as adjunct to chemotherapy); stimulation or growth of bone, cartilage, tendons, ligaments and/or nerves (e.g. for treating wounds, stimulation of follicle stimulating hormone (for control of fertility); chemotactic and chemokinetic activities (e.g. for treating infections, tumors); hemostatic or thrombolytic activity (e.g. for treating hemophilia, cardiac infarction etc.); anti-inflammatory activity (e.g. for treating septic shock, Crohn's disease); as antimicrobials; for treating psoriasis or other hyperproliferative diseases; for regulation of metabolism, and behavior. Also contemplated is the use of the corresponding nucleic acid in gene therapy procedures. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their

interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:48 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2595 of SEQ ID NO:48, b is an integer of 15 to 2609, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:48, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 39

This gene is expressed primarily in placenta, prostate and neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immune and endocrine disorders, as well as, disorders of developing systems. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, developing system and endocrine system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in placenta suggests that the protein product of this clone is useful for the diagnosis and/or treatment of disorders of the placenta. Specific expression within the placenta suggests that this gene product may play a role in the proper establishment and maintenance of placental function. Alternately, this gene product may be produced by the placenta and then transported to the embryo, where it may play a crucial role in the development and/or survival of the developing embryo or fetus. Expression of this gene product in a vascular-rich tissue such as the placenta also suggests that this gene product may be produced more generally in endothelial cells or within the circulation. In such instances, it may play more generalized roles in vascular function, such as in angiogenesis. It may also be produced in the vasculature and have effects on other cells within the circulation, such as hematopoietic cells. It may serve to promote the proliferation, survival, activation, and/or differentiation of hematopoietic cells, as well as other cells throughout the body. The expression in prostate may indicate the gene or its products can be used in the disorders of the prostate, including inflammatory disorders, such as chronic prostatitis, granulomatous prostatitis and malacoplakia, prostatic hyperplasia and prostate neoplastic disorders, including adenocarcinoma, transitional cell carcinomas, ductal carcinomas, squamous cell carcinomas, or as hormones or factors with systemic or reproductive functions.

The tissue distribution in neutrophils indicates the protein product of this clone is useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated

cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:49 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1884 of SEQ ID NO:49, b is an integer of 15 to 1898, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:49, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 40

The gene encoding the disclosed cDNA is believed to reside on chromosome 8. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 8.

This gene is expressed primarily in HL-60 myeloid leukemia cell line, uterus, ovarian tumor, synovium, lung, brain and to a lesser extent in wide variety of human tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: myeloid leukemia, ovarian cancer and disorders of the central nervous system (CNS). Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system and CNS expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in immune cells indicates the protein product of this clone is useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as

autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

The tissue distribution in brain indicates the protein product of this clone is useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:50 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1794 of SEQ ID NO:50, b is an integer of 15 to 1808, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:50, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 41

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence: HDTRLPLPGQHGRGAWVCLTVLVCSTVDSNDSLYGGDSKFLAENNKLCET VMAQILEHLKTLAKDEALKRQSSLGLSFFNSILAHGDLRNNKLNQLSVNLWH LAQRHGCADTRTMVKTLE YIKKQSKQPDMTHLTELALRLPLQTRT SEQ ID NO: 185(SEQ ID NO) Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in fibroblasts, retina, multiple sclerosis, testes, fetal tissue, synovial sarcoma, and hepatoma and to a lesser extent in many other tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: wound healing/connective tissue disorders, endocrine disorders, eye disorders, synovium and liver cancers or tumors of other origins. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological

probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the synovium, fibroblasts, retina, testes, and liver expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 123 as residues: Ser-33 to Thr-44. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in testes indicates the protein product of this clone would be useful for the detection, treatment, and/or prevention of various endocrine disorders and cancers. Representative uses are described in the "Biological Activity", "Hyperproliferative Disorders", and "Binding Activity" sections below, in Example 11, 17, 18, 19, 20 and 27, and elsewhere herein. Briefly, the protein can be used for the detection, treatment, and/or prevention of Addison’s disease, Cushing’s Syndrome, and disorders and/or cancers of the pancrease (e.g. diabetes mellitus), adrenal cortex, ovaries, pituitary (e.g., hyper-, hypopituitarism), thyroid (e.g. hyper-, hypothyroidism), parathyroid (e.g. hyper-,hypoparathyroidism) , hypothallamus, and testes. Moreover, the expression within fetal tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of

potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:51 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 941 of SEQ ID NO:51, b is an integer of 15 to 955, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:51, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 42

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

MLFVDSGSTRLRKKTLSGDFIFMNRCQSSRQPRPAGVNKHLWGCPASSRTSH EWLLWPKAVLQAKQTALGWNSPT (SEQ ID NO: 186), CQSSRQPRPAGVNKHLWGCPASSRTSHEWLLWPKAVLQAKQTALGWNSPT (SEQ ID NO: 187), KWGCFCKGSSFTPHSCPPEAPLFPAVLLVSTLG (SEQ ID NO: 188), and CPPEAPLFPAVLLVSTLG (SEQ ID NO: 189). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in endometrial tumor, kidney, fetal tissue, uterine cancer, skin cancer, pancreas and to a lesser extent in many other tissues

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: fetal development disorders, disorders of the endocrine and exocrine system, cancers of the endometrium, uterus, skin and cancer, in general. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine and exocrine system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 124 as residues:

Arg-66 to Gly-74. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in immune cells indicates the protein product of this clone is useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

The tissue distribution in pancreas and kidney suggests that the protein product of this clone is useful for the detection, treatment, and/or prevention of various endocrine disorders and cancers, particularly Addison's disease, Cushing's Syndrome, and disorders and/or cancers of the pancrease (e.g. diabetes mellitus), adrenal cortex, ovaries, pituitary (e.g., hyper-, hypopituitarism), thyroid (e.g. hyper-, hypothyroidism), parathyroid (e.g. hyper-, hypoparathyroidism) , hypothallamus, and testes. Furthermore, the protein may also be used to determine biological activity,

raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:52 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1833 of SEQ ID NO:52, b is an integer of 15 to 1847, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:52, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 43

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 148-164 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 165-253 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ia membrane proteins.

This gene is expressed primarily in brain, immune cells, testes and to a lesser extent in many other tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the central nervous system (CNS), testes, and immune disorders Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell

type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, CNS, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, neural, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 125 as residues: Glu-34 to Leu-46, Glu-58 to Asn-65, Pro-93 to Glu-98, Pro-122 to Ser-127. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in brain indicates the protein product of this clone is useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival.

The tissue distribution in immune cells (e.g., T-cells) indicates the protein product of this clone is useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in

regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. The tissue distribution in testes tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of male reproductive and endocrine disorders. It may also prove to be valuable in the diagnosis and treatment of testicular cancer, as well as cancers of other tissues where expression has been observed. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:53 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2149 of SEQ ID NO:53, b is an integer of 15 to 2163, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:53, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 44

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence:
EGADKMATSVGHRCLGLLHGVAPWRSSLHPCEITALSQSLQPLRKLPFRAFR
TDARKIHTAPARTMFLLRPLPILLVTGGGYAGYRQYEKYRERELEKLGLEIPP
KLAGHWEVALYKSVPTRLLSRAWGRLNQVELPH WLRRPVYSLYIWTXGG
(SEQ ID NO: 190) Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to these polypeptides, or polypeptides encoded by a polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention and polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in synovial sarcoma, retina, fetal tissue, brain, amd immune cells (e.g., T-cells).

Polynucleotides and polypeptides of the invention would be useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell

types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 126 as residues: Gln-22 to Leu-31. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in brain indicates the protein product of this clone is useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival.

The tissue distribution in T-cells indicates the protein product of this clone is useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation

of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Moreover, the expression within fetal tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types

of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:54 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 734 of SEQ ID NO:54, b is an integer of 15 to 748, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:54, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 45

This gene is expressed primarily in tumors of the parathyroid gland, skin, prostate and colon. .

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: integumentary, reproductive, and endocrine diseases and/or disorders, particularly cancers of the prostate, skin, parathyroid and colon. Similarly, polypeptides and

antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the prostate, skin, parathyroid and colon expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., integumentary, reproductive, gastrointestinal, endocrine, prostate, skin, colon, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in skin indicates that polynucleotides and polypeptides corresponding to this gene would be useful for treatment, prevention, detection and/or diagnosis of cancers of the prostate, skin, parathyroid and colon. Representative uses are described in the "Biological Activity", "Hyperproliferative Disorders", "Infectious Disease", and "Regeneration" sections below, in Example 11, 19, and 20, and elsewhere herein. Briefly, the protein is useful in detecting, treating, and/or preventing congenital disorders (i.e., nevi, moles, freckles, Mongolian spots, hemangiomas, port-wine syndrome), integumentary tumors (i.e., keratoses, Bowen's disease, basal cell carcinoma, squamous cell carcinoma, malignant melanoma, Paget's disease, mycosis fungoides, and Kaposi's sarcoma), injuries and inflammation of the skin (i.e., wounds, rashes, prickly heat disorder, psoriasis, dermatitis), atherosclerosis, uticaria, eczema, photosensitivity, autoimmune disorders (i.e., lupus erythematosus, vitiligo, dermatomyositis, morphea, scleroderma, pemphigoid, and pemphigus), keloids, striae, erythema, petechiae, purpura, and xanthelasma. In addition, such disorders may predispose increased susceptibility to viral and bacterial infections of the skin (i.e., cold sores, warts, chickenpox, molluscum contagiosum, herpes zoster, boils, cellulitis, erysipelas, impetigo, tinea, althletes foot, and ringworm). Moreover, polynucleotides and/or polypeptides of the invention may also be useful for the treatment, prevention, detection and/or diagnosis of various connective tissue disorders (i.e., arthritis, trauma, tendonitis, chrondomalacia and inflammation, etc.), autoimmune disorders (i.e., rheumatoid arthritis, lupus, scleroderma, dermatomyositis, etc.), dwarfism, spinal deformation, joint abnormalities, amd

chondrodysplasias (i.e., spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). Expression in prostate tissue indicates the gene or its products would be useful for diagnosis, treatment and/or prevention of the disorders of the prostate, including inflammatory disorders, such as chronic prostatitis, granulomatous prostatitis and malacoplakia, prostatic hyperplasia and prostate neoplastic disorders, including adenocarcinoma, transitional cell carcinomas, ductal carcinomas, squamous cell carcinomas, or as hormones or factors with systemic or reproductive functions. In addition, polynucleotides and/or polypeptides corresponding to this gene would be useful in the treatment of male infertility, and/or could be used as a male contraceptive. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:55 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1184 of SEQ ID NO:55, b is an integer of 15 to 1198, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:55, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 46

This gene is expressed primarily in fibroblasts, placenta, pancreas, brain, monocytes and to a lesser extent in many other tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immune system and/or neurodegenerative disorders, including but not limited to brain disorders Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, neural, nervous, neuronal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 128 as residues: Ala-62 to Ser-87. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in brain indicates that polynucleotides and/or polypeptides corresponding to this clone would be useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in

normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival.

In addition, the tissue distribution in immune tissues indicates that polynucleotides and/or polypeptides corresponding to this gene would be useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:56 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 953 of SEQ ID NO:56, b is an integer of 15 to 967, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:56, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 47

The translation product of this gene shares sequence homology with motilin which has gastrointestinal motor stimulating activity and binds with high affinity to the motilin receptor and mimics the peristaltic effects of motilin on gastrointestinal tissue.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: REQLSCFSSHTWCPWEGVLWAPQAQGVMSAPPPHPQPPAAPTSRNYTEIREK LRSRLTRRKEELPMKGGTLGGIPGEPAVDHRDVDELLEFINSTEPKVPNSARA AKRARHKLKKKVGVGRAQLCRLSSLRTLAPTPRTSGA (SEQ ID NO: 191) and ARGSGQGEEAVQKSHKVKRRGPLVRVEQLRIEEMKVIKLLVTFELGVIILILE MTKLRLTKTR (SEQ ID NO: 192). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in brain frontal cortex.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of central nervous system and gastrointestinal disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the digestive system, CNS, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 129 as residues: Pro-41 to Thr-46, Cys-48 to Gly-59, Pro-79 to Trp-84, Ala-86 to Gly-94. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The homology to motilin indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of gastrointestinal disorders, such as malabsorption, diarrheal diseases, gastroenteritis, tumors, colitis and bowel diseases. The tissue distribution in brain indicates the protein product of this clone is useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS,

psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:57 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1133 of SEQ ID NO:57, b is an integer of 15 to 1147, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:57, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 48

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:
TLLKGTKLELHRGGGRSRTSGSPGLQEFGTRPTPGVWSCPTATPWASGSRRK
NLARESKGRPRPTEITRPYLCPHPYLPPHTAPCLGSHPSACRCSRSCPHSLLLPF
SITRECPGSHRVPQMPVFPQTILSSRINSIAIQMSPHQPMQVSSSKTILWLVLSC
LCPSSPHPVISGLPQWYIGVLAGIVPVAPIRPGDSGLDLQREGPQPIL
SQGLNRRT (SEQ ID NO: 193). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at

least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in immune cells (e.g., T-cells) and to a lesser extent in breast cancer, kidney, and ovary.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immune disorders and breast cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 130 as residues: Met-1 to Pro-6, Gly-73 to Thr-78. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in T-cells indicates the protein product of this clone is useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation

of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:58 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 961 of SEQ ID NO:58, b is an integer of 15 to 975, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:58, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 49

The translation product of this gene shares sequence homology with alpha mannosidases thought to be important in oligosaccharide processing (see, e.g., Genbank Accession No. gb|AAA82446.1, and Geneseq Accession No. W48265; all information and references available through these accessions are hereby incorporated herein by reference). Based on the sequence similarity, the translation product of this clone is expected to share at least some biological activities with mannosidase proteins. Such activities are known in the art, some of which are described elsewhere herein.

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence:
VDGAAMAACEGRRSGALGSSQSDFLTPPVGGAPWAVATTVVMYPPPPPPH
RDFISVTLSFGESYDNSKSWRRRSCWRKWKQLSRLQRNMILFLLAFLLFCGLL
FYINLADHWKALAFRLEEEQKMRPEIAGLKPANPPVLPAPQKADTDPENLPEI
SSQKTQRHIQRGPPHLQIRPPSQDLKDGTQEEATKRQEAPVDPRPEGDPQRTV
ISWRGAVIEPEQGTELPSRRAEVPTKPPLPPARTQGTPVHLNYRQKGVIDVFL
HAWKGYRKFAWGHDELKPVSRSFSEWFGLGLTLIDALDTMWILGLRKEFEE
ARKWVSKKLHFEKDVDVNLFESTIRILGGLLSAYHLSGDSLFLRKAEDFGNRL
MPAFRTPSKIPYSDVNIGTGVAHPPRWTSDSTVAEVTSIQLEFRELSRLTGDKK
FQEAVEKVTQHIHGLSGKKDGLVPMFINTHSGLFTHLGVFTLGARADSYYEY
LLKQWIQGGKQETQLLEDYVEAIEGVRTHLLRHSEPSKLTFVGELAHGRFSA
KMDHLVCFLPGTLALGVYHGLPASHMELAQELMETCYQMNRQMETGLSPEI
VHFNLYPQPGRRDVEVKPADRHNLLRPETVESLFYLYRVTGDRKYQDWGWE
ILQSFSRFTRVPSGGYSSINNVQDPQKPEPRDKMESFFLGETLKYLFLLFSDDP
NLLSLDAYVFNTEAHPLPIWTPA (SEQ ID NO:194). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to these polypeptides, or polypeptides encoded by a polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are

encompassed by the invention. Antibodies that bind polypeptides of the invention and polynucleotides encoding these polypeptides are also encompassed by the invention.

When tested against human T cells, supernatants removed from cells expressing this gene induced expression of the secreted cytokine, IL-13.

An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines, e.g.TNF-alpha, IL-1, IL-10, IL-12. Thus, it is likely that the product of this gene is involved in the activation of T cells, in addition to other immune cell-lines or immune tissue cell types. Accordingly, polynucleotides and polypeptides related to this gene may have uses which include, but are not limited to, activating immune cells, such as during an inflammatory response.

This gene is expressed primarily in endocrine organs but also in normal and transformed cell types from other tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: metabolic, infectious, and growth diseases, disorders, and defects, including cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine organs, and/or immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., endocrine, metabolic, immune, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 131 as residues: Glu-32 to Arg-38, Gln-56 to Asn-64, Ser-69 to His-83, Arg-87 to Gln-118, Leu-137 to Thr-146, Pro-148 to Gly-157, Trp-177 to Ala-184, Asp-188 to Ser-194, Lys-221 to Arg-227, Arg-283 to Pro-289, Pro-302 to Asp-308, Thr-328 to Phe-333, Ser-348 to

Gly-353, Gly-392 to Leu-400, Arg-416 to Lys-422, Tyr-493 to Glu-502, Thr-527 to Trp-535, Asn-559 to Met-572. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in endocrine tissues, combined with the homology to mannosidases indicates that polynucleotides and polypeptides corresponding to this gene would be useful for study, prevention, detection, diagnosis and/or treatment of hormonal, metabolic and immune/host defense disorders and neoplasms. The protein product of this clone would be useful for the detection, treatment, and/or prevention of various endocrine disorders and cancers. Representative uses are described in the "Biological Activity", "Hyperproliferative Disorders", and "Binding Activity" sections below, in Example 11, 17, 18, 19, 20 and 27, and elsewhere herein. Briefly, the protein can be used for the detection, treatment, and/or prevention of Addison's disease, Cushing's Syndrome, and disorders and/or cancers of the pancrease (e.g., diabetes mellitus), adrenal cortex, ovaries, pituitary (e.g., hyper-, hypopituitarism), thyroid (e.g., hyper-, hypothyroidism), parathyroid (e.g., hyper-,hypoparathyroidism) , hypothallamus, and testes. Based upon the strong homology to mannosidases, the protein is likely to be useful in correcting secretory protein defects at the level of protein metabolism. Moreover, antagonists of this protein would be useful in the treatment of rapidly proliferating cells and tissues, including cancers. The protein, including variants thereof, could also be useful in creating novel glycosylated proteins. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:59 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

general formula of a-b, where a is any integer between 1 to 2719 of SEQ ID NO:59, b is an integer of 15 to 2733, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:59, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 50

This gene is expressed primarily in immune (e.g., dendritic cells and B-cells), haemopoietic, and fetal cells and to a lesser extent in several other tissues and cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immune and haemopoietic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and haemopoietic system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in immune cells indicates the protein product of this clone is useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in

immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

The expression within fetal tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in

modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:60 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1654 of SEQ ID NO:60, b is an integer of 15 to 1668, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:60, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 51

The translation product of this gene shares sequence homology with the complement C1q A chain precursor (See Genbank Accession No. gb|AAD32626.1; in addition to the following Geneseq Accession Nos. Y01481 and Y12319; all information contained within these accessions in combination with the references referred to therein are hereby incorporated herein by reference). The present invention is believed to represent a novel splice variant of the complement C1q A chain precursor protein.

This gene is expressed primarily in primary dendritic cells, breast lymph node, colon tumor, normal colon, human adult pulmonary, and to a lesser extent, in ulcerative colitis, thymus, bone marrow, and human adipose.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immune and hematopoietic diseases and/or disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune or gastrointestinal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, gastrointestinal, pulmonary, metabolic, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 133 as residues: Pro-29 to Gly-46, Lys-48 to Gly-55, Lys-67 to Gly-80, Gly-89 to Asn-99. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in hematopoietic cells and tissues, combined with the homology to complement C1q A chain precursor indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment, detection, and/or prevention of various immune and hematopoietic diseases and/or disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia,

rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:61 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1007 of SEQ ID NO:61, b is an integer of 15 to 1021, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:61, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 52

This gene is expressed primarily in fetal liver spleen, cem cells/cyclohexamide treated, and to a lesser extent in glioblastoma cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immune, hematopoietic, developmental, and hepatic diseases and/or disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoietic system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, developmental, hepatic, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, amniotic fluid, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 134 as residues: Gln-30 to Gly-38. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in fetal/liver spleen indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment, detection, and/or prevention of immune, hemapoietic, and developmental diseases and/or disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis,

acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:62 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 899 of SEQ ID NO:62, b is an integer of 15 to 913, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:62, and where b is greater than or equal to a + 14.

Table 1

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
1	HETHR73	PTA-623 09/02/99	Uni-ZAP XR	11	2219	1	2219	207	207	83	1	44	45	563
2	HDPFB02	PTA-622 09/02/99	pCMVSport 3.0	12	3436	1	3436	173	173	84	1	19	20	152
2	HDPFB02	PTA-622 09/02/99	pCMVSport 3.0	63	1517	1	1517	139	139	135	1	28	29	316
2	HMWDB84	PTA-622 09/02/99	Uni-ZAP XR	64	2751	1	2751	218	218	136	1	18	19	302
3	HNTEO78	PTA-623 09/02/99	pCMVSport 3.0	13	734	1	734	89	89	85	1	18	19	215
4	HDPFY41	PTA-622 09/02/99	pCMVSport 3.0	14	5330	1	5330	158	158	86	1	25	26	831
5	HDPIE85	PTA-622 09/02/99	pCMVSport 3.0	15	2753	1	2753	57	57	87	1	25	26	480
6	HDPOE32	PTA-622 09/02/99	pCMVSport 3.0	16	1353	1	1353	118	118	88	1	34	35	151
7	HLQEM64	PTA-623 09/02/99	Lambda ZAP II	17	1038	1	702	42	42	89	1	29	30	132
8	HNGIR58	PTA-623 09/02/99	Uni-ZAP XR	18	718	1	718	52	52	90	1	33	34	110

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
8	HMAMI21	PTA-725 09/20/99	Uni-ZAP XR	65	2150	1	2150	13	13	137	1	1	2	374
9	HOEEK12	PTA-623 09/02/99	Uni-ZAP XR	19	1198	80	1152	131	131	91	1	21	22	188
9	HJPAY76	209852 05/07/98	Uni-ZAP XR	66	1161	1	1161	134	134	138	1	21	22	127
10	HTLIT63	PTA-623 09/02/99	Uni-ZAP XR	20	1033	1	1033	249	249	92	1	23	24	179
11	HNEBY54	PTA-622 09/02/99	Uni-ZAP XR	21	1732	1	1732	176	176	93	1	21	22	259
12	HFKKS66	PTA-623 09/02/99	Uni-ZAP XR	22	840	1	830	7	7	94	1	39	40	239
13	HFVJP07	PTA-623 09/02/99	pBluescript	23	940	1	940	108	108	95	1	18	19	138
14	HTEAM34	PTA-623 09/02/99	Uni-ZAP XR	24	801	87	801	136	136	96	1	28	29	122
14	HTEAM34	209463 11/14/97	Uni-ZAP XR	67	734	1	734	63	63	139	1	28	29	122
15	HUFGH53	PTA-623 09/02/99	pSport1	25	1969	1	1969	36	36	97	1	22	23	459
16	HMADJ14	PTA-622 09/02/99	Uni-ZAP XR	26	1364	15	1364	278	278	98	1	68	69	352

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
16	HMADJ14	PTA-622 09/02/99	Uni-ZAP XR	68	1583	1	1583	264	264	140	1	26	27	257
16	HMADJ14	PTA-622 09/02/99	Uni-ZAP XR	69	1444	91	1444	125	125	141	1	26	27	257
16	HMADJ74	PTA-622 09/02/99	Uni-ZAP XR	70	1892	619	1855	264	264	142	1	38	39	291
16	HMABG70	209076 05/22/97	Uni-ZAP XR	71	1439	18	1316	276	276	143	1	19	20	21
17	HETAY39	PTA-622 09/02/99	Uni-ZAP XR	27	2371	1	2371	54	54	99	1	24	25	257
18	HFPFK57	PTA-623 09/02/99	Uni-ZAP XR	28	867	1	867	78	78	100	1	19	20	127
19	HSICO66	PTA-622 09/02/99	Uni-ZAP XR	29	1605	1	1605	176	176	101	1	20	21	136
20	HUFBC44	PTA-622 09/02/99	pSport1	30	1334	1	1334	87	87	102	1	20	21	144
21	HAAAI67	PTA-622 09/02/99	pSport1	31	1011	1	1011	62	62	103	1	19	20	151
21	HFKIA71	PTA-736 09/21/99	Uni-ZAP XR	72	1395	213	1361	349	349	144	1	41	42	173
22	HOSNU69	PTA-623 09/02/99	Uni-ZAP XR	32	1308	269	1142	408	408	104	1	32	33	112

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
23	HMSCM88	PTA-622 09/02/99	Uni-ZAP XR	33	1434	1	1434	236	236	105	1	25	26	80
24	HSXAZ05	PTA-622 09/02/99	Uni-ZAP XR	34	2184	1	2184	125	125	106	1	27	28	51
25	HTPCW21	PTA-622 09/02/99	Uni-ZAP XR	35	1296	1	1296	171	171	107	1	35	36	60
25	HTPCW21	PTA-622 09/02/99	Uni-ZAP XR	73	1293	1	1293	171	171	145	1	35	36	60
26	HNGOW62	PTA-622 09/02/99	Uni-ZAP XR	36	1298	1	1298	167	167	108	1	19	20	54
27	HAVVG36	PTA-622 09/02/99	Other	37	553	1	553	139	139	109	1	25	26	97
28	HBGNP63	PTA-622 09/02/99	Uni-ZAP XR	38	601	1	601	203	203	110	1	48	49	122
29	HNHNB29	PTA-623 09/02/99	Uni-ZAP XR	39	1894	1	1894	40	40	111	1	20	21	53
30	HPJCL28	PTA-623 09/02/99	Uni-ZAP XR	40	3279	1	3279	309	309	112	1	29	30	80
31	HOFNC14	PTA-623 09/02/99	pCMVSport 2.0	41	3095	1	3095	155	155	113	1	13	14	72
32	HELHN47	PTA-622 09/02/99	Uni-ZAP XR	42	2320	1	2320	102	102	114	1	36	37	45

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
32	HELHN47	PTA-622 09/02/99	Uni-ZAP XR	74	3147	842	3147	935	935	146	1	36	37	45
32	HELHN47	PTA-622 09/02/99	Uni-ZAP XR	75	1989	883	1989	778	778	147	1	30	31	404
33	HFKET18	PTA-622 09/02/99	Uni-ZAP XR	43	2407	1	2407	137	137	115	1	14	15	74
34	HSRFZ57	PTA-622 09/02/99	Uni-ZAP XR	44	1930	1	1925	82	82	116	1	18	19	41
35	HAMFP32	PTA-622 09/02/99	pCMVSport 3.0	45	1459	1	1459	247	247	117	1	20	21	82
36	HLHDL42	PTA-622 09/02/99	Uni-ZAP XR	46	1003	1	1001	198	198	118	1	20	21	53
36	HAPQU71	PTA-181 06/07/99	Uni-ZAP XR	76	1879	1264	1807	1324	1324	148	1	20	21	53
36	HAPQU71	203917 04/08/99	Uni-ZAP XR	77	1879	1264	1807	1324	1324	149	1	20	21	53
37	HKMLX18	PTA-622 09/02/99	pBluescript	47	1358	1	1358	154	154	119	1	27	28	180
38	HFIUW36	PTA-623 09/02/99	pSport1	48	2609	1	2609	319	319	120	1	1	2	599
39	HNGOU82	PTA-622 09/02/99	Uni-ZAP XR	49	1898	1	1646	83	83	121	1	24	25	45

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
40	HSODB85	PTA-622 09/02/99	Uni-ZAP XR	50	1808	1	1808	509	509	122	1	21	22	57
41	HFICR14	PTA-622 09/02/99	pSport1	51	955	1	955	272	272	123	1	29	30	50
41	HFICR14	PTA-499 08/11/99	pSport1	78	955	1	955	272	272	150	1	29	30	50
42	HSIDQ93	PTA-622 09/02/99	Uni-ZAP XR	52	1847	1	1847	155	155	124	1	26	27	74
43	HSLGM81	PTA-622 09/02/99	Uni-ZAP XR	53	2163	1	2163	248	248	125	1	20	21	253
43	HSYBM41	PTA-987 11/24/99	pCMVSport 3.0	79	2309	89	2092	129	129	151	1	20	21	253
44	HLQGP82	PTA-623 09/02/99	Lambda ZAP II	54	748	29	748	202	202	126	1	18	19	89
44	HSSDG41	209076 05/22/97	Uni-ZAP XR	80	2619	25	2080	202	202	152	1	18	19	127
45	HRACI26	PTA-623 09/02/99	pCMVSport 3.0	55	1198	1	1198	55	55	127	1	19	20	49
46	HMSMD07	PTA-623 09/02/99	Uni-ZAP XR	56	967	1	967	450	450	128	1	18	19	90
47	HFXDK20	PTA-622 09/02/99	Lambda ZAP II	57	1147	1	1147	348	348	129	1	27	28	94

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
48	HTXKF95	PTA-622 09/02/99	Uni-ZAP XR	58	975	170	966	421	421	130	1	28	29	78
48	HTXKF95	PTA-622 09/02/99	Uni-ZAP XR	81	884	79	875	330	330	153	1	28	29	78
49	HUSBA88	PTA-623 09/02/99	Lambda ZAP II	59	2733	27	2733	270	270	131	1	15	16	615
50	HNEDD37	PTA-622 09/02/99	Uni-ZAP XR	60	1668	1	1668	29	29	132	1	19	20	42
51	HBJNC59	PTA-622 09/02/99	Uni-ZAP XR	61	1021	1	1021	66	66	133	1	22	23	99
51	HAPQT56	PTA-909 11/02/99	Uni-ZAP XR	82	1086	45	1012	64	64	154	1	22	23	245
52	HCABW07	PTA-622 09/02/99	Uni-ZAP XR	62	913	1	913	33	33	134	1	31	32	57

Table 1 summarizes the information corresponding to each "Gene No." described above. The nucleotide sequence identified as "NT SEQ ID NO:X" was assembled from partially homologous ("overlapping") sequences obtained from the "cDNA clone ID" identified in Table 1 and, in some cases, from additional related DNA clones. The overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:X.

The cDNA Clone ID was deposited on the date and given the corresponding deposit number listed in "ATCC Deposit No:Z and Date." Some of the deposits contain multiple different clones corresponding to the same gene. "Vector" refers to the type of vector contained in the cDNA Clone ID.

"Total NT Seq." refers to the total number of nucleotides in the contig identified by "Gene No." The deposited clone may contain all or most of these sequences, reflected by the nucleotide position indicated as "5' NT of Clone Seq." and the "3' NT of Clone Seq." of SEQ ID NO:X. The nucleotide position of SEQ ID NO:X of the putative start codon (methionine) is identified as "5' NT of Start Codon." Similarly , the nucleotide position of SEQ ID NO:X of the predicted signal sequence is identified as "5' NT of First AA of Signal Pep."

The translated amino acid sequence, beginning with the methionine, is identified as "AA SEQ ID NO:Y," although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

The first and last amino acid position of SEQ ID NO:Y of the predicted signal peptide is identified as "First AA of Sig Pep" and "Last AA of Sig Pep." The predicted first amino acid position of SEQ ID NO:Y of the secreted portion is identified as "Predicted First AA of Secreted Portion." Finally, the amino acid position of SEQ ID NO:Y of the last amino acid in the open reading frame is identified as "Last AA of ORF."

SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing) and the translated SEQ ID NO:Y (where Y may be any of the polypeptide sequences disclosed in the sequence listing) are sufficiently

accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:X is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in the deposited clone. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used, for example, to generate antibodies which bind specifically to proteins containing the polypeptides and the secreted proteins encoded by the cDNA clones identified in Table 1.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X and the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing a human cDNA of the invention deposited with the ATCC, as set forth in Table 1. The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or the deposited clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed

herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or a deposited clone, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

Table 2 summarizes the expression profile of polynucleotides corresponding to the clones disclosed in Table 1. The first column provides a unique clone identifier, "Clone ID", for a cDNA clone related to each contig sequence disclosed in Table 1. Column 2, "Library Code" shows the expression profile of tissue and/or cell line libraries which express the polynucleotides of the invention. Each Library Code in column 2 represents a tissue/cell source identifier code corresponding to the Library Code and Library description provided in Table 4. Expression of these polynucleotides was not observed in the other tissues and/or cell libraries tested. One of skill in the art could routinely use this information to identify tissues which show a predominant expression pattern of the corresponding polynucleotide of the invention or to identify polynucleotides which show predominant and/or specific tissue expression.

Table 3, column 1, provides a nucleotide sequence identifier, "SEQ ID NO:X," that matches a nucleotide SEQ ID NO:X disclosed in Table 1, column 5. Table 3, column 2, provides the chromosomal location, "Cytologic Band or Chromosome," of polynucleotides corresponding to SEQ ID NO:X. Chromosomal location was determined by finding exact matches to EST and cDNA sequences contained in the NCBI (National Center for Biotechnology Information) UniGene database. Given a presumptive chromosomal location, disease locus association was

determined by comparison with the Morbid Map, derived from Online Mendelian Inheritance in Man (Online Mendelian Inheritance in Man, OMIM™. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD) 2000. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/). If the putative chromosomal location of the Query overlapped with the chromosomal location of a Morbid Map entry, the OMIM reference identification number of the morbid map entry is provided in Table 3, column 3, labelled "OMIM ID." A key to the OMIM reference identification numbers is provided in Table 5.

Table 4 provides a key to the Library Code disclosed in Table 2. Column 1 provides the Library Code disclosed in Table 2, column 2. Column 2 provides a description of the tissue or cell source from which the corresponding library was derived.

Table 5 provides a key to the OMIM reference identification numbers disclosed in Table 3, column 3. OMIM reference identification numbers (Column 1) were derived from Online Mendelian Inheritance in Man (Online Mendelian Inheritance in Man, OMIM. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine, (Bethesda, MD) 2000. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/). Column 2 provides diseases associated with the cytologic band disclosed in Table 3, column 2, as determined using the Morbid Map database.

Table 2

Clone ID	Library Codes
HETHR73	H0031 H0039 H0046 H0059 H0144 H0150 H0164 H0180 H0181 H0196 H0208 H0213 H0253 H0254 H0255 H0266 H0271 H0309 H0333 H0381 H0383 H0413 H0424 H0427 H0441 H0445 H0486 H0488 H0506 H0518 H0521 H0529 H0538 H0539 H0553 H0555 H0575 H0581 H0587 H0599 H0606 H0617 H0619 H0620 H0634 H0638 H0644 H0647 H0658 L0105 L0362 L0499 L0581 L0603 L0605 L0612 L0659 L0662 L0666 L0731 L0743 L0747 L0749 L0751 L0770 L0775 L0789 L0794 L0800 L0809 S0001 S0027 S0028 S0031 S0037 S0038 S0044 S0045 S0046 S0049 S0050 S0052 S0116 S0126 S0152 S0222 S0278 S0308 S0360 S0364 S0376 S0390 S0428 T0004 T0048 T0110
HDPFB02	H0039 H0050 H0056 H0059 H0063 H0123 H0124 H0131 H0132 H0135 H0246 H0250 H0252 H0265 H0292 H0295 H0341 H0355 H0391 H0393 H0413 H0478 H0494 H0519 H0520 H0521 H0522 H0529 H0539 H0545 H0546 H0547 H0551 H0553 H0561 H0580 H0586 H0606 H0628 H0633 H0658 H0662 H0668 H0670 H0672 H0686 H0696 L0386 L0439 L0523 L0581 L0595 L0597 L0638 L0645 L0650 L0651 L0658 L0659 L0663 L0666 L0731 L0740 L0744 L0747 L0748 L0751 L0755 L0756 L0757 L0759 L0761 L0766 L0768 L0769 L0770 L0772 L0774 L0775 L0776 L0777 L0779 L0783 L0806 L0809 S0002 S0028 S0040 S0046 S0049 S0126 S0144 S0212 S0222 S0294 S0354 S0358 S0376 S0388 S0418 S0420
HNTEO78	H0519 H0638 H0653 L0731 L0750 L0755 L0770 L0771 L0773 L0774 L0779
HDPFY41	H0008 H0144 H0163 H0409 H0428 H0486 H0521 H0615 H0624 H0658 H0661 H0662 H0672 L0375 L0439 L0591 L0602 L0649 L0650 L0655 L0659 L0661 L0662 L0665 L0666 L0717 L0731 L0754 L0756 L0759 L0766 L0777 L0779 L0791 L0792 L0803 L0806 S0003 S0011 S0036 S0114 S0152 S0356 S0426 S6024
HDPIE85	H0046 H0050 H0124 H0144 H0208 H0266 H0286 H0288 H0411 H0412 H0445 H0486 H0521 H0539 H0542 H0547 H0549 H0555 H0560 H0575 H0599 H0616 H0622 H0623 H0624 H0634 H0660 H0682 L0363 L0439 L0455 L0471 L0564 L0565 L0591 L0592 L0599 L0602 L0605 L0635 L0645 L0659 L0662 L0663 L0664 L0731 L0740 L0747 L0748 L0750 L0754 L0757 L0758 L0759 L0766 L0777 L0779 L0783 L0789 L0790 L0794 S0027 S0028 S0031 S0038 S0052 S0126 S0192 S0212 S0222 S0242 S0250 S0276 S0282 S0360 S0390 S0418 S0420 S3014 S6028 T0060
HDPOE32	H0039 H0040 H0052 H0264 H0331 H0343 H0422 H0435 H0506 H0522 H0529 H0530 H0543 H0551 H0556 H0591 H0620 L0005 L0521 L0581 L0740 L0751 L0752 S0046 S0152 T0042
HLQEM64	H0370 H0393 H0412 H0413 H0510 H0551 H0574 H0581 H0696 L0595 L0663 L0754 L0794 S0212
HNGIR58	S0052
HOEEK12	H0011 H0014 H0031 H0038 H0041 H0052 H0083 H0085 H0123 H0135 H0144 H0255 H0266 H0341 H0412 H0415 H0458 H0510 H0529 H0543 H0545 H0556 H0560 H0587 H0618 H0619 H0620 H0626 H0631 L0371 L0471 L0526 L0655 L0659 L0663 L0747 L0749 L0750 L0751 L0757 L0758 L0764 L0766 L0769 L0773 L0774 L0776 L0779 L0780 L0784 L0786 L0803 L0804 L0809 S0126 S0144 S0294 S0328 S0358 S0360 S0366 S0374 S0408 S3014 T0008

HTLIT63	H0253 H0618 L0758 L0794
HNEBY54	H0040 H0069 H0179 H0265 H0280 H0416 H0423 H0486 H0529 H0542 H0559 H0617 H0634 H0635 H0637 H0647 H0657 H0658 H0682 H0688 L0021 L0372 L0608 L0626 L0638 L0646 L0662 L0666 L0731 L0749 L0751 L0758 L0759 L0768 L0771 L0777 L0788 L0794 L0800 L0803 L0809 S0027 S0038 S0044 S0046 S0051 S0116 S0142 S0364 T0004 T0060
HFKKS66	H0085 H0125 H0135 H0253 H0341 H0424 H0494 H0543 H0556 H0559 H0561 H0618 H0620 H0633 H0634 H0637 L0595 L0606 L0645 L0649 L0653 L0662 L0663 L0731 L0747 L0749 L0752 L0754 L0757 L0758 L0761 L0766 L0769 L0772 L0773 L0776 L0777 L0779 L0780 L0787 L0788 L0794 L0805 L0806 L0809 S0002 S0051 S0134 S0212 S0328 S0330 S0388 S0420 T0023
HFVJP07	H0370 H0393 L0748 L0755 L0780
HTEAM34	H0038 H0616 H0618 L0758 L0794
HUFGH53	H0012 H0059 H0087 H0483 H0506 H0529 H0540 H0617 H0622 H0624 H0625 H0660 H0672 H0687 L0617 L0639 L0663 L0731 L0747 L0750 L0752 L0755 L0764 L0769 L0783 L0787 L0794 L0800 L0803 L0809 S0038 S0354 S0374 S0418 S6026
HMADJ14	H0068 H0364 H0521 H0575 H0591 H0638 L0776 L0791 L0806 S0002 S0003 S0122 S0144 S0214 S0278 S0344
HETAY39	H0015 H0046 H0105 H0318 H0352 H0369 H0478 H0494 H0510 H0549 H0596 H0598 H0615 H0622 H0689 L0439 L0631 L0640 L0647 L0659 L0662 L0663 L0664 L0665 L0666 L0752 L0758 L0763 L0779 L0780 L0789 L0805 L0809 S0146
HFPFK57	H0670 L0375 L0439 L0601 L0653 L0731 L0749 L0750 L0751 L0759 L0769 L0775 L0777 L0779 L0800 L0803 L0809 S0222 S0364
HSICO66	H0004 H0009 H0024 H0036 H0038 H0040 H0041 H0046 H0052 H0056 H0059 H0063 H0068 H0083 H0087 H0090 H0100 H0135 H0188 H0216 H0252 H0255 H0265 H0271 H0318 H0352 H0370 H0392 H0393 H0402 H0421 H0422 H0423 H0428 H0436 H0437 H0484 H0486 H0494 H0506 H0520 H0521 H0522 H0543 H0547 H0551 H0555 H0556 H0561 H0581 H0587 H0595 H0598 H0599 H0602 H0615 H0616 H0617 H0618 H0638 H0641 H0657 H0658 H0659 H0660 H0670 H0677 H0686 H0690 L0002 L0163 L0357 L0362 L0369 L0372 L0375 L0382 L0438 L0471 L0483 L0485 L0499 L0513 L0515 L0523 L0595 L0596 L0600 L0601 L0639 L0646 L0650 L0653 L0655 L0657 L0659 L0663 L0664 L0665 L0666 L0731 L0740 L0741 L0747 L0748 L0749 L0750 L0751 L0752 L0754 L0755 L0757 L0758 L0761 L0763 L0764 L0766 L0768 L0769 L0770 L0771 L0772 L0774 L0776 L0777 L0779 L0787 L0791 L0794 L0800 L0803 L0809 S0001 S0002 S0010 S0026 S0028 S0051 S0132 S0142 S0150 S0152 S0218 S0278 S0344 S0348 S0354 S0358 S0360 S0374 S0424 S3014 T0006 T0041 T0049 T0109
HUFBC44	H0144 H0478 H0506 L0372 L0438 L0589 L0601 L0659 L0665 L0763 L0769 L0791 S0222
HAAAI67	H0024 H0030 H0086 H0122 H0165 H0170 H0171 H0181 H0188 H0213 H0222 H0255 H0294 H0309 H0333 H0373 H0411 H0436 H0444 H0484 H0485 H0486 H0521 H0540 H0542 H0543 H0545 H0547 H0555 H0560 H0561 H0580 H0583 H0587 H0617 H0620 H0638 H0646 H0650 H0656 H0657 H0658 H0661 H0664 H0670 H0672 H0674 H0677 H0682 H0683 H0689 L0055 L0157 L0382 L0529 L0542 L0581 L0601 L0622 L0623 L0638 L0655 L0657 L0659 L0662 L0665 L0666 L0717 L0731 L0740 L0742 L0743 L0747 L0750 L0751 L0753 L0754 L0755 L0757 L0758 L0759 L0764 L0766 L0768 L0769 L0770 L0776 L0777 L0803 L0809 S0022 S0026 S0052 S0132 S0142 S0176 S0194 S0250 S0358 S0360 S0374 S0390 S0420 S0434 S6024 T0006 T0010 T0040

HOSNU69	H0222 H0341 H0370 H0423 H0529 H0543 H0581 H0591 H0619 H0662 L0526 L0533 L0558 L0770 L0771 S0003 S0360 S0424
HMSCM88	S0002
HSXAZ05	H0024 L0809 S0007 S0010 S0036 S0049 S0051
HTPCW21	H0039 H0052 H0478
HNGOW62	H0556 S0428
HAVVG36	H0038 H0051 H0251 H0413 H0423 H0509 H0543 H0551 H0553 H0652 H0665 H0670 H0672 H0682 L0021 L0055 L0485 L0545 L0662 L0664 L0731 L0748 L0752 L0754 L0756 L0758 L0764 L0766 L0767 L0779 L0794 L0803 L0806 S0192 S0196 S0214 S0356 S0360 S0414
HBGNP63	H0617
HNHNB29	S0216
HPJCL28	L0589 L0779 S0031 S0152
HOFNC14	H0415
HELHN47	H0009 H0013 H0031 H0032 H0050 H0052 H0057 H0059 H0090 H0131 H0201 H0265 H0266 H0318 H0339 H0344 H0393 H0409 H0413 H0423 H0441 H0445 H0458 H0522 H0538 H0561 H0566 H0581 H0593 H0672 H0707 L0352 L0366 L0438 L0439 L0455 L0456 L0471 L0493 L0542 L0591 L0636 L0637 L0648 L0650 L0653 L0659 L0665 L0666 L0731 L0740 L0745 L0747 L0748 L0749 L0750 L0751 L0752 L0756 L0758 L0759 L0761 L0764 L0766 L0769 L0774 L0776 L0779 L0783 L0789 S0005 S0007 S0028 S0045 S0046 S0126 S0134 S0136 S0150 S0152 S0212 S0222 S0348 S0360 S0364 S0366 S0386 S0388 S0422 S0426 S0446 T0003 T0041 T0060 T0069 T0082
HFKET18	H0012 H0013 H0087 H0100 H0107 H0183 H0188 H0255 H0265 H0318 H0341 H0352 H0445 H0486 H0529 H0543 H0544 H0549 H0556 H0583 H0597 H0617 H0618 H0619 H0620 H0644 H0660 H0674 H0690 L0055 L0438 L0439 L0646 L0657 L0663 L0666 L0717 L0731 L0740 L0747 L0750 L0751 L0756 L0758 L0759 L0761 L0763 L0764 L0766 L0768 L0769 L0771 L0774 L0794 L0804 L0809 S0028 S0046 S0182 S0358 S0360 S0402 S0418 S0456
HSRFZ57	S0014 S0022
HAMFP32	H0042 H0063 H0265 H0266 H0373 H0484 H0486 H0489 H0542 H0543 H0547 H0556 H0560 H0594 H0617 H0618 H0637 H0685 H0698 L0371 L0655 L0766 L0770 L0774 L0777 L0789 L0803 L0804 L0805 L0809 S0028 S0116 S0192 S0218 S0328 S0360 S0418 S0420 S0436 S3012
HLHDL42	H0024 H0042 H0046 H0477 H0575 H0658 H0663 H0670 H0672 H0685 H0690 L0527 L0599 L0639 L0657 L0662 L0664 L0665 L0666 L0751 L0755 L0775 L0806 S0016
HKMLX18	H0002 H0013 H0014 H0031 H0038 H0046 H0052 H0123 H0144 H0187 H0194 H0264 H0266 H0294 H0341 H0352 H0423 H0431 H0436 H0494 H0506 H0519 H0521 H0529 H0538 H0539 H0547 H0553 H0556 H0598 H0615 H0616 H0623 H0631 H0634 H0641 H0657 H0672 H0687 L0375 L0438 L0471 L0600 L0638 L0646 L0655 L0659 L0663 L0666 L0731 L0740 L0743 L0745 L0747 L0748 L0749 L0750 L0752 L0755 L0757 L0758 L0759 L0761 L0763 L0766 L0771 L0773 L0774 L0777 L0790 L0794 L0803 L0804 L0805 L0809 S0010 S0028 S0046 S0126 S0150 S0152 S0222 S0242 S0280 S0328 S0344 S0356 S0364 S0378 S0422 S6024
HFIUW36	H0013 H0039 H0046 H0083 H0090 H0265 H0327 H0423 H0521 H0539 H0555 H0574 H0581 H0599 H0615 H0622 H0628 H0631 H0649 H0656 H0694 L0002 L0438 L0439 L0455 L0456 L0520 L0637 L0648 L0664 L0666 L0667 L0731 L0740 L0743 L0747 L0748 L0752 L0754 L0756 L0758 L0761 L0763 L0766 L0770 L0776 L0779 L0789 L0794 L0800 L0803 L0809 S0002 S0026 S0049 S0196 S0222 S0250 S0280 S0424

	T0041
HNGOU82	H0030 S0150 S0428
HSODB85	H0004 H0251 H0294 H0331 H0341 H0427 H0489 H0497 H0509 H0521 H0586 H0591 H0595 H0615 H0624 H0632 H0633 H0646 H0648 H0665 L0194 L0352 L0369 L0438 L0439 L0471 L0480 L0591 L0599 L0608 L0637 L0640 L0641 L0646 L0659 L0662 L0665 L0666 L0731 L0740 L0744 L0747 L0751 L0752 L0755 L0761 L0763 L0764 L0766 L0773 L0774 L0775 L0777 L0792 L0803 L0805 L0806 L0809 S0002 S0003 S0040 S0152 S0196 S0212 S0214 S0328 S0330 S0356 S0360 S0376 S0380 S0418 T0114
HFICR14	H0038 H0040 H0052 H0135 H0265 H0316 H0333 H0486 H0509 H0521 H0522 H0542 H0547 H0555 H0599 H0618 H0647 H0672 H0684 H0689 L0040 L0361 L0455 L0471 L0518 L0542 L0559 L0565 L0637 L0640 L0651 L0659 L0665 L0747 L0748 L0749 L0752 L0756 L0766 L0768 L0769 L0770 L0774 L0776 L0779 L0787 L0790 L0793 L0803 L0805 L0809 S0010 S0028 S0040 S0192 S0378 S0420 T0023
HSIDQ93	H0011 H0036 H0046 H0059 H0068 H0392 H0444 H0620 H0672 L0517 L0649 L0659 L0717 L0747 L0748 L0754 L0777 L0794 S0114 S0222 S0330 S0428 T0023
HSLGM81	H0009 H0013 H0031 H0038 H0050 H0051 H0052 H0144 H0156 H0201 H0253 H0284 H0341 H0373 H0393 H0435 H0445 H0455 H0519 H0521 H0538 H0542 H0547 H0551 H0553 H0575 H0581 H0583 H0586 H0591 H0622 H0632 H0633 H0697 H0701 L0351 L0352 L0438 L0439 L0592 L0630 L0731 L0742 L0748 L0752 L0758 L0769 L0775 L0776 L0789 L0791 N0005 S0007 S0010 S0028 S0031 S0036 S0045 S0049 S0052 S0112 S0126 S0134 S0222 S0282 S0310 S0346 S3012 T0039 T0041
HLQGP82	H0135 H0484 H0494 H0553 H0561 H0622 H0632 H0667 H0677 L0741 L0745 L0746 L0748 S0038 S0222 S0440
HRACI26	H0555 L0520 L0659 L0740 L0752 L0766 L0773 L0779 S0210 S0356
HMSMD07	H0009 H0014 H0040 H0052 H0056 H0081 H0250 H0261 H0264 H0266 H0268 H0286 H0427 H0441 H0445 H0478 H0506 H0510 H0521 H0538 H0544 H0551 H0553 H0575 H0581 H0586 H0593 H0600 H0620 H0623 H0644 H0648 H0662 H0670 L0021 L0163 L0351 L0372 L0438 L0439 L0483 L0523 L0581 L0637 L0646 L0659 L0662 L0731 L0743 L0744 L0747 L0748 L0749 L0757 L0770 L0774 L0775 L0776 L0777 L0783 L0789 L0790 L0791 L0794 L0800 L0803 L0804 S0010 S0022 S0027 S0028 S0032 S0036 S0038 S0040 S0045 S0051 S0126 S0210 S0276 S0278 S0282 S0330 S0354 S0360 S0426 S0474 S3014 T0010 T0040
HFXDK20	H0436 S0001
HTXKF95	H0030 H0050 H0051 H0124 H0144 H0265 H0305 H0422 H0441 H0506 H0543 H0553 H0555 H0556 H0569 H0586 H0599 H0616 L0363 L0471 L0599 L0603 L0605 L0644 L0659 L0662 L0665 L0666 L0731 L0747 L0748 L0749 L0750 L0751 L0754 L0755 L0764 L0769 L0770 L0775 L0779 L0783 L0794 L0800 L0803 L0804 L0806 S0046 S0358 S3012
HUSBA88	H0013 H0014 H0024 H0039 H0040 H0052 H0059 H0071 H0100 H0123 H0124 H0135 H0144 H0150 H0156 H0170 H0194 H0208 H0213 H0231 H0251 H0253 H0255 H0264 H0265 H0269 H0329 H0333 H0352 H0370 H0371 H0392 H0393 H0413 H0455 H0479 H0483 H0486 H0494 H0518 H0520 H0521 H0522 H0529 H0539 H0544 H0545 H0547 H0550 H0553 H0555 H0556 H0581 H0594 H0607 H0616 H0617 H0632 H0633 H0641 H0644 H0645 H0647 H0649 H0651 H0653 H0658 H0659 H0660 H0661 H0663 H0666 H0667 H0670 H0672 H0673 H0678 H0684 H0686 H0687

	H0689 H0690 H0696 L0021 L0040 L0163 L0351 L0374 L0378 L0384 L0439 L0462 L0527 L0549 L0592 L0596 L0602 L0605 L0619 L0637 L0639 L0644 L0645 L0657 L0659 L0663 L0664 L0665 L0666 L0698 L0717 L0731 L0740 L0742 L0745 L0747 L0748 L0749 L0750 L0751 L0752 L0753 L0754 L0757 L0758 L0759 L0761 L0763 L0764 L0765 L0766 L0768 L0769 L0770 L0771 L0772 L0773 L0774 L0775 L0777 L0779 L0783 L0794 L0796 L0800 L0803 L0806 L0809 S0002 S0005 S0010 S0027 S0028 S0038 S0040 S0051 S0114 S0126 S0132 S0140 S0146 S0152 S0194 S0206 S0212 S0222 S0276 S0334 S0358 S0360 S0374 S0380 S0390 S0418 S0420 S0426 S0444 S0448 S3014 T0041 T0067
HNEDD37	H0179 H0197 H0271 H0309 H0436 H0522 H0549 H0550 H0650 L0517 L0745 L0752 L0758 S0106 S0114
HBJNC59	H0009 H0015 H0030 H0031 H0039 H0042 H0045 H0087 H0100 H0120 H0124 H0252 H0254 H0255 H0309 H0318 H0327 H0352 H0375 H0411 H0421 H0424 H0427 H0445 H0455 H0506 H0509 H0510 H0521 H0522 H0538 H0550 H0555 H0575 H0581 H0583 H0587 H0602 H0617 H0632 H0637 H0638 H0641 H0647 H0649 H0653 H0661 H0663 H0672 H0687 H0689 L0375 L0378 L0385 L0540 L0545 L0547 L0603 L0629 L0636 L0644 L0648 L0651 L0653 L0655 L0657 L0659 L0747 L0749 L0750 L0754 L0755 L0762 L0763 L0767 L0768 L0769 L0772 L0774 L0775 L0776 L0777 L0783 L0806 S0044 S0116 S0260 S0280 S0292 S0332 S0356 S0358 S0360 S0374 S0376 S0380 S0404 S6022 T0082
HCABW07	H0125 H0351 L0748

Table 3

SEQ ID NO: X	Cytologic Band or Chromosome:	OMIM Reference(s):
25	22q12.1-q12.2	101000 123620 138981 188826 600850 601669
59	9	

Table 4

Library Code	Library Description
H0002	Human Adult Heart
H0004	Human Adult Spleen
H0008	Whole 6 Week Old Embryo
H0009	Human Fetal Brain
H0011	Human Fetal Kidney
H0012	Human Fetal Kidney
H0013	Human 8 Week Whole Embryo
H0014	Human Gall Bladder
H0015	Human Gall Bladder, fraction II
H0024	Human Fetal Lung III
H0030	Human Placenta
H0031	Human Placenta
H0032	Human Prostate
H0036	Human Adult Small Intestine
H0038	Human Testes
H0039	Human Pancreas Tumor
H0040	Human Testes Tumor
H0041	Human Fetal Bone
H0042	Human Adult Pulmonary
H0045	Human Esophagus, Cancer
H0046	Human Endometrial Tumor
H0050	Human Fetal Heart
H0051	Human Hippocampus
H0052	Human Cerebellum
H0056	Human Umbilical Vein, Endo. remake
H0057	Human Fetal Spleen
H0059	Human Uterine Cancer
H0063	Human Thymus
H0068	Human Skin Tumor
H0069	Human Activated T-Cells
H0071	Human Infant Adrenal Gland
H0081	Human Fetal Epithelium (Skin)
H0083	HUMAN JURKAT MEMBRANE BOUND POLYSOMES
H0085	Human Colon
H0086	Human epithelioid sarcoma
H0087	Human Thymus
H0090	Human T-Cell Lymphoma
H0100	Human Whole Six Week Old Embryo
H0105	Human Fetal Heart, subtracted
H0107	Human Infant Adrenal Gland, subtracted
H0120	Human Adult Spleen, subtracted
H0122	Human Adult Skeletal Muscle
H0123	Human Fetal Dura Mater
H0124	Human Rhabdomyosarcoma
H0125	Cem cells cyclohexamide treated
H0131	LNCAP + o.3nM R1881
H0132	LNCAP + 30nM R1881

H0135	Human Synovial Sarcoma
H0144	Nine Week Old Early Stage Human
H0150	Human Epididymus
H0156	Human Adrenal Gland Tumor
H0163	Human Synovium
H0164	Human Trachea Tumor
H0165	Human Prostate Cancer, Stage B2
H0170	12 Week Old Early Stage Human
H0171	12 Week Old Early Stage Human, II
H0179	Human Neutrophil
H0180	Human Primary Breast Cancer
H0181	Human Primary Breast Cancer
H0183	Human Colon Cancer
H0187	Resting T-Cell
H0188	Human Normal Breast
H0194	Human Cerebellum, subtracted
H0196	Human Cardiomyopathy, subtracted
H0197	Human Fetal Liver, subtracted
H0201	Human Hippocampus, subtracted
H0208	Early Stage Human Lung, subtracted
H0213	Human Pituitary, subtracted
H0216	Supt cells, cyclohexamide treated, subtracted
H0222	Activated T-Cells, 8 hrs, subtracted
H0231	Human Colon, subtraction
H0246	Human Fetal Liver- Enzyme subtraction
H0250	Human Activated Monocytes
H0251	Human Chondrosarcoma
H0252	Human Osteosarcoma
H0253	Human adult testis, large inserts
H0254	Breast Lymph node cDNA library
H0255	breast lymph node CDNA library
H0261	H. cerebellum, Enzyme subtracted
H0264	human tonsils
H0265	Activated T-Cell (12hs)/Thiouridine labelledEco
H0266	Human Microvascular Endothelial Cells, fract. A
H0268	Human Umbilical Vein Endothelial Cells, fract. A
H0269	Human Umbilical Vein Endothelial Cells, fract. B
H0271	Human Neutrophil, Activated
H0280	K562 + PMA (36 hrs)
H0284	Human OB MG63 control fraction I
H0286	Human OB MG63 treated (10 nM E2) fraction I
H0288	Human OB HOS control fraction I
H0292	Human OB HOS treated (10 nM E2) fraction I
H0294	Amniotic Cells - TNF induced
H0295	Amniotic Cells - Primary Culture
H0305	CD34 positive cells (Cord Blood)
H0309	Human Chronic Synovitis
H0316	HUMAN STOMACH
H0318	HUMAN B CELL LYMPHOMA
H0327	human corpus colosum
H0329	Dermatofibrosarcoma Protuberance
H0331	Hepatocellular Tumor
H0333	Hemangiopericytoma

H0339	Duodenum
H0341	Bone Marrow Cell Line (RS4,11)
H0343	stomach cancer (human)
H0344	Adipose tissue (human)
H0351	Glioblastoma
H0352	wilm's tumor
H0355	Human Liver
H0364	Human Osteoclastoma, excised
H0369	H. Atrophic Endometrium
H0370	H. Lymph node breast Cancer
H0371	Eosinophils-Hypereosinophilia patient
H0373	Human Heart
H0375	Human Lung
H0381	Bone Cancer
H0383	Human Prostate BPH, re-excision
H0391	H. Meniingima, M6
H0392	H. Meningima, M1
H0393	Fetal Liver, subtraction II
H0402	CD34 depleted Buffy Coat (Cord Blood), re-excision
H0409	H. Striatum Depression, subtracted
H0411	H Female Bladder, Adult
H0412	Human umbilical vein endothelial cells, IL-4 induced
H0413	Human Umbilical Vein Endothelial Cells, uninduced
H0415	H. Ovarian Tumor, II, OV5232
H0416	Human Neutrophils, Activated, re-excision
H0421	Human Bone Marrow, re-excision
H0422	T-Cell PHA 16 hrs
H0423	T-Cell PHA 24 hrs
H0424	Human Pituitary, subt IX
H0427	Human Adipose
H0428	Human Ovary
H0431	H. Kidney Medulla, re-excision
H0435	Ovarian Tumor 10-3-95
H0436	Resting T-Cell Library,II
H0437	H Umbilical Vein Endothelial Cells, frac A, re-excision
H0441	H. Kidney Cortex, subtracted
H0444	Spleen metastic melanoma
H0445	Spleen, Chronic lymphocytic leukemia
H0455	H. Striatum Depression, subt
H0458	CD34+ cell, I, frac II
H0477	Human Tonsil, Lib 3
H0478	Salivary Gland, Lib 2
H0479	Salivary Gland, Lib 3
H0483	Breast Cancer cell line, MDA 36
H0484	Breast Cancer Cell line, angiogenic
H0485	Hodgkin's Lymphoma I
H0486	Hodgkin's Lymphoma II
H0488	Human Tonsils, Lib 2
H0489	Crohn's Disease
H0494	Keratinocyte
H0497	HEL cell line
H0506	Ulcerative Colitis
H0509	Liver, Hepatoma

H0510	Human Liver, normal
H0518	pBMC stimulated w/ poly I/C
H0519	NTERA2, control
H0520	NTERA2 + retinoic acid, 14 days
H0521	Primary Dendritic Cells, lib 1
H0522	Primary Dendritic cells,frac 2
H0529	Myoloid Progenitor Cell Line
H0530	Human Dermal Endothelial Cells,untreated
H0538	Merkel Cells
H0539	Pancreas Islet Cell Tumor
H0540	Skin, burned
H0542	T Cell helper I
H0543	T cell helper II
H0544	Human endometrial stromal cells
H0545	Human endometrial stromal cells-treated with progesterone
H0546	Human endometrial stromal cells-treated with estradiol
H0547	NTERA2 teratocarcinoma cell line+retinoic acid (14 days)
H0549	H. Epididiymus, caput & corpus
H0550	H. Epididiymus, cauda
H0551	Human Thymus Stromal Cells
H0553	Human Placenta
H0555	Rejected Kidney, lib 4
H0556	Activated T-cell(12h)/Thiouridine-re-excision
H0559	HL-60, PMA 4H, re-excision
H0560	KMH2
H0561	L428
H0566	Human Fetal Brain,normalized c50F
H0569	Human Fetal Brain, normalized CO
H0574	Hepatocellular Tumor, re-excision
H0575	Human Adult Pulmonary,re-excision
H0580	Dendritic cells, pooled
H0581	Human Bone Marrow, treated
H0583	B Cell lymphoma
H0586	Healing groin wound, 6.5 hours post incision
H0587	Healing groin wound, 7.5 hours post incision
H0591	Human T-cell lymphoma,re-excision
H0593	Olfactory epithelium,nasalcavity
H0594	Human Lung Cancer,re-excision
H0595	Stomach cancer (human),re-excision
H0596	Human Colon Cancer,re-excision
H0597	Human Colon, re-excision
H0598	Human Stomach,re-excision
H0599	Human Adult Heart,re-excision
H0600	Healing Abdomen wound,70&90 min post incision
H0602	Healing Abdomen Wound,21&29 days post incision
H0606	Human Primary Breast Cancer,re-excision
H0607	H.Leukocytes, normalized cot 50A3
H0615	Human Ovarian Cancer Reexcision
H0616	Human Testes, Reexcision
H0617	Human Primary Breast Cancer Reexcision
H0618	Human Adult Testes, Large Inserts, Reexcision
H0619	Fetal Heart
H0620	Human Fetal Kidney, Reexcision

H0622	Human Pancreas Tumor, Reexcision
H0623	Human Umbilical Vein, Reexcision
H0624	12 Week Early Stage Human II, Reexcision
H0625	Ku 812F Basophils Line
H0626	Saos2 Cells, Untreated
H0628	Human Pre-Differentiated Adipocytes
H0631	Saos2, Dexamethosome Treated
H0632	Hepatocellular Tumor,re-excision
H0633	Lung Carcinoma A549 TNFalpha activated
H0634	Human Testes Tumor, re-excision
H0635	Human Activated T-Cells, re-excision
H0637	Dendritic Cells From CD34 Cells
H0638	CD40 activated monocyte dendridic cells
H0641	LPS activated derived dendritic cells
H0644	Human Placenta (re-excision)
H0645	Fetal Heart, re-excision
H0646	Lung, Cancer (4005313 A3): Invasive Poorly Differentiated Lung Adenocarcinoma,
H0647	Lung, Cancer (4005163 B7): Invasive, Poorly Diff. Adenocarcinoma, Metastatic
H0648	Ovary, Cancer: (4004562 B6) Papillary Serous Cystic Neoplasm, Low Malignant Pot
H0649	Lung, Normal: (4005313 B1)
H0650	B-Cells
H0651	Ovary, Normal: (9805C040R)
H0652	Lung, Normal: (4005313 B1)
H0653	Stromal Cells
H0656	B-cells (unstimulated)
H0657	B-cells (stimulated)
H0658	Ovary, Cancer (9809C332): Poorly differentiated adenocarcinoma
H0659	Ovary, Cancer (15395A1F): Grade II Papillary Carcinoma
H0660	Ovary, Cancer: (15799A1F) Poorly differentiated carcinoma
H0661	Breast, Cancer: (4004943 A5)
H0662	Breast, Normal: (4005522B2)
H0663	Breast, Cancer: (4005522 A2)
H0664	Breast, Cancer: (9806C012R)
H0665	Stromal cells 3.88
H0666	Ovary, Cancer: (4004332 A2)
H0667	Stromal cells(HBM3.18)
H0668	stromal cell clone 2.5
H0670	Ovary, Cancer(4004650 A3): Well-Differentiated Micropapillary Serous Carcinoma
H0672	Ovary, Cancer: (4004576 A8)
H0673	Human Prostate Cancer, Stage B2, re-excision
H0674	Human Prostate Cancer, Stage C, re-excission
H0677	TNFR degenerate oligo
H0678	screened clones from placental library
H0682	Ovarian cancer, Serous Papillary Adenocarcinoma
H0683	Ovarian cancer, Serous Papillary Adenocarcinoma
H0684	Ovarian cancer, Serous Papillary Adenocarcinoma
H0685	Adenocarcinoma of Ovary, Human Cell Line, # OVCAR-3
H0686	Adenocarcinoma of Ovary, Human Cell Line
H0687	Human normal ovary(#9610G215)
H0688	Human Ovarian Cancer(#9807G017)
H0689	Ovarian Cancer
H0690	Ovarian Cancer, # 9702G001
H0694	Prostate cancer (adenocarcinoma)

H0696	Prostate Adenocarcinoma
H0697	NK Cells (NKYao20 Control)
H0698	NK CellsYao20 IL2 treated for 48 hrs
H0701	NKyao15(control)
H0707	Stomach Cancer(S007635)
L0002	Atrium cDNA library Human heart
L0005	Clontech human aorta polyA+ mRNA (#6572)
L0021	Human adult (K.Okubo)
L0040	Human colon mucosa
L0055	Human promyelocyte
L0105	Human aorta polyA+ (TFujiwara)
L0157	Human fetal brain (TFujiwara)
L0163	Human heart cDNA (YNakamura)
L0194	Human pancreatic cancer cell line Patu 8988t
L0351	Infant brain, Bento Soares
L0352	Normalized infant brain, Bento Soares
L0357	V, Human Placenta tissue
L0361	Stratagene ovary (#937217)
L0362	Stratagene ovarian cancer (#937219)
L0363	NCI_CGAP_GC2
L0366	Stratagene schizo brain S11
L0369	NCI_CGAP_AA1
L0371	NCI_CGAP_Br3
L0372	NCI_CGAP_Co12
L0374	NCI_CGAP_Co2
L0375	NCI_CGAP_Kid6
L0378	NCI_CGAP_Lu1
L0382	NCI_CGAP_Pr25
L0384	NCI_CGAP_Pr23
L0385	NCI_CGAP_Gas1
L0386	NCI_CGAP_HN3
L0438	normalized infant brain cDNA
L0439	Soares infant brain 1NIB
L0455	Human retina cDNA randomly primed sublibrary
L0456	Human retina cDNA Tsp509I-cleaved sublibrary
L0462	WATM1
L0471	Human fetal heart, Lambda ZAP Express
L0480	Stratagene cat#937212 (1992)
L0483	Human pancreatic islet
L0485	STRATAGENE Human skeletal muscle cDNA library, cat. #936215.
L0493	NCI_CGAP_Ov26
L0499	NCI_CGAP_HSC2
L0513	NCI_CGAP_Ov37
L0515	NCI_CGAP_Ov32
L0517	NCI_CGAP_Pr1
L0518	NCI_CGAP_Pr2
L0520	NCI_CGAP_Alv1
L0521	NCI_CGAP_Ew1
L0523	NCI_CGAP_Lip2
L0526	NCI_CGAP_Pr12
L0527	NCI_CGAP_Ov2
L0529	NCI_CGAP_Pr6
L0533	NCI_CGAP_HSC1

L0540	NCI_CGAP_Pr10
L0542	NCI_CGAP_Pr11
L0545	NCI_CGAP_Pr4.1
L0547	NCI_CGAP_Pr16
L0549	NCI_CGAP_HN10
L0558	NCI_CGAP_Ov40
L0559	NCI_CGAP_Ov39
L0564	Jia bone marrow stroma
L0565	Normal Human Trabecular Bone Cells
L0581	Stratagene liver (#937224)
L0589	Stratagene fetal retina 937202
L0591	Stratagene HeLa cell s3 937216
L0592	Stratagene hNT neuron (#937233)
L0595	Stratagene NT2 neuronal precursor 937230
L0596	Stratagene colon (#937204)
L0597	Stratagene corneal stroma (#937222)
L0599	Stratagene lung (#937210)
L0600	Weizmann Olfactory Epithelium
L0601	Stratagene pancreas (#937208)
L0602	Pancreatic Islet
L0603	Stratagene placenta (#937225)
L0605	Stratagene fetal spleen (#937205)
L0606	NCI_CGAP_Lym5
L0608	Stratagene lung carcinoma 937218
L0612	Schiller oligodendroglioma
L0617	Chromosome 22 exon
L0619	Chromosome 9 exon II
L0622	HM1
L0623	HM3
L0626	NCI_CGAP_GC1
L0629	NCI_CGAP_Mel3
L0630	NCI_CGAP_CNS1
L0631	NCI_CGAP_Br7
L0635	NCI_CGAP_PNS1
L0636	NCI_CGAP_Pit1
L0637	NCI_CGAP_Brn53
L0638	NCI_CGAP_Brn35
L0639	NCI_CGAP_Brn52
L0640	NCI_CGAP_Br18
L0641	NCI_CGAP_Co17
L0644	NCI_CGAP_Co20
L0645	NCI_CGAP_Co21
L0646	NCI_CGAP_Co14
L0647	NCI_CGAP_Sar4
L0648	NCI_CGAP_Eso2
L0649	NCI_CGAP_GU1
L0650	NCI_CGAP_Kid13
L0651	NCI_CGAP_Kid8
L0653	NCI_CGAP_Lu28
L0655	NCI_CGAP_Lym12
L0657	NCI_CGAP_Ov23
L0658	NCI_CGAP_Ov35
L0659	NCI_CGAP_Pan1

L0661	NCI_CGAP_Mel15
L0662	NCI_CGAP_Gas4
L0663	NCI_CGAP_Ut2
L0664	NCI_CGAP_Ut3
L0665	NCI_CGAP_Ut4
L0666	NCI_CGAP_Ut1
L0667	NCI_CGAP_CML1
L0698	Testis 2
L0717	Gessler Wilms tumor
L0731	Soares_pregnant_uterus_NbHPU
L0740	Soares melanocyte 2NbHM
L0741	Soares adult brain N2b4HB55Y
L0742	Soares adult brain N2b5HB55Y
L0743	Soares breast 2NbHBst
L0744	Soares breast 3NbHBst
L0745	Soares retina N2b4HR
L0746	Soares retina N2b5HR
L0747	Soares_fetal_heart_NbHH19W
L0748	Soares fetal liver spleen 1NFLS
L0749	Soares_fetal_liver_spleen_1NFLS_S1
L0750	Soares_fetal_lung_NbHL19W
L0751	Soares ovary tumor NbHOT
L0752	Soares_parathyroid_tumor_NbHPA
L0753	Soares_pineal_gland_N3HPG
L0754	Soares placenta Nb2HP
L0755	Soares_placenta_8to9weeks_2NbHP8to9W
L0756	Soares_multiple_sclerosis_2NbHMSP
L0757	Soares_senescent_fibroblasts_NbHSF
L0758	Soares_testis_NHT
L0759	Soares_total_fetus_Nb2HF8_9w
L0761	NCI_CGAP_CLL1
L0762	NCI_CGAP_Br1.1
L0763	NCI_CGAP_Br2
L0764	NCI_CGAP_Co3
L0765	NCI_CGAP_Co4
L0766	NCI_CGAP_GCB1
L0767	NCI_CGAP_GC3
L0768	NCI_CGAP_GC4
L0769	NCI_CGAP_Brn25
L0770	NCI_CGAP_Brn23
L0771	NCI_CGAP_Co8
L0772	NCI_CGAP_Co10
L0773	NCI_CGAP_Co9
L0774	NCI_CGAP_Kid3
L0775	NCI_CGAP_Kid5
L0776	NCI_CGAP_Lu5
L0777	Soares_NhHMPu_S1
L0779	Soares_NFL_T_GBC_S1
L0780	Soares_NSF_F8_9W_OT_PA_P_S1
L0783	NCI_CGAP_Pr22
L0784	NCI_CGAP_Lei2
L0786	Soares_NbHFB
L0787	NCI_CGAP_Sub1

L0788	NCI_CGAP_Sub2
L0789	NCI_CGAP_Sub3
L0790	NCI_CGAP_Sub4
L0791	NCI_CGAP_Sub5
L0792	NCI_CGAP_Sub6
L0793	NCI_CGAP_Sub7
L0794	NCI_CGAP_GC6
L0796	NCI_CGAP_Brn50
L0800	NCI_CGAP_Co16
L0803	NCI_CGAP_Kid11
L0804	NCI_CGAP_Kid12
L0805	NCI_CGAP_Lu24
L0806	NCI_CGAP_Lu19
L0809	NCI_CGAP_Pr28
N0005	Human cerebral cortex
S0001	Brain frontal cortex
S0002	Monocyte activated
S0003	Human Osteoclastoma
S0005	Heart
S0007	Early Stage Human Brain
S0010	Human Amygdala
S0011	STROMAL -OSTEOCLASTOMA
S0014	Kidney Cortex
S0016	Kidney Pyramids
S0022	Human Osteoclastoma Stromal Cells - unamplified
S0026	Stromal cell TF274
S0027	Smooth muscle, serum treated
S0028	Smooth muscle,control
S0031	Spinal cord
S0032	Smooth muscle-ILb induced
S0036	Human Substantia Nigra
S0037	Smooth muscle, IL1b induced
S0038	Human Whole Brain #2 - Oligo dT > 1.5Kb
S0040	Adipocytes
S0044	Prostate BPH
S0045	Endothelial cells-control
S0046	Endothelial-induced
S0049	Human Brain, Striatum
S0050	Human Frontal Cortex, Schizophrenia
S0051	Human Hypothalmus,Schizophrenia
S0052	neutrophils control
S0106	STRIATUM DEPRESSION
S0112	Hypothalamus
S0114	Anergic T-cell
S0116	Bone marrow
S0122	Osteoclastoma-normalized A
S0126	Osteoblasts
S0132	Epithelial-TNFa and INF induced
S0134	Apoptotic T-cell
S0136	PERM TF274
S0140	eosinophil-IL5 induced
S0142	Macrophage-oxLDL
S0144	Macrophage (GM-CSF treated)

S0146	prostate-edited
S0150	LNCAP prostate cell line
S0152	PC3 Prostate cell line
S0176	Prostate, normal, subtraction I
S0182	Human B Cell 8866
S0192	Synovial Fibroblasts (control)
S0194	Synovial hypoxia
S0196	Synovial IL-1/TNF stimulated
S0206	Smooth Muscle- HASTE normalized
S0210	Messangial cell, frac 2
S0212	Bone Marrow Stromal Cell, untreated
S0214	Human Osteoclastoma, re-excision
S0216	Neutrophils IL-1 and LPS induced
S0218	Apoptotic T-cell, re-excision
S0222	H. Frontal cortex,epileptic,re-excision
S0242	Synovial Fibroblasts (Il1/TNF), subt
S0250	Human Osteoblasts II
S0260	Spinal Cord, re-excision
S0276	Synovial hypoxia-RSF subtracted
S0278	H Macrophage (GM-CSF treated), re-excision
S0280	Human Adipose Tissue, re-excision
S0282	Brain Frontal Cortex, re-excision
S0292	Osteoarthritis (OA-4)
S0294	Larynx tumor
S0308	Spleen/normal
S0310	Normal trachea
S0328	Palate carcinoma
S0330	Palate normal
S0332	Pharynx carcinoma
S0334	Human Normal Cartilage Fraction III
S0344	Macrophage-oxLDL, re-excision
S0346	Human Amygdala,re-excision
S0348	Cheek Carcinoma
S0354	Colon Normal II
S0356	Colon Carcinoma
S0358	Colon Normal III
S0360	Colon Tumor II
S0364	Human Quadriceps
S0366	Human Soleus
S0374	Normal colon
S0376	Colon Tumor
S0378	Pancreas normal PCA4 No
S0380	Pancreas Tumor PCA4 Tu
S0386	Human Whole Brain, re-excision
S0388	Human Hypothalamus,schizophrenia, re-excision
S0390	Smooth muscle, control, re-excision
S0402	Adrenal Gland,normal
S0404	Rectum normal
S0408	Colon, normal
S0414	Hippocampus, Alzheimer Subtracted
S0418	CHME Cell Line,treated 5 hrs
S0420	CHME Cell Line,untreated
S0422	Mo7e Cell Line GM-CSF treated (1ng/ml)

S0424	TF-1 Cell Line GM-CSF Treated
S0426	Monocyte activated, re-excision
S0428	Neutrophils control, re-excision
S0434	Stomach Normal
S0436	Stomach Tumour
S0440	Liver Tumour Met 5 Tu
S0444	Colon Tumor
S0446	Tongue Tumour
S0448	Larynx Normal
S0456	Tongue Normal
S0474	Human blood platelets
S3012	Smooth Muscle Serum Treated, Norm
S3014	Smooth muscle, serum induced,re-exc
S6022	H. Adipose Tissue
S6024	Alzheimers, spongy change
S6026	Frontal Lobe, Dementia
S6028	Human Manic Depression Tissue
T0003	Human Fetal Lung
T0004	Human White Fat
T0006	Human Pineal Gland
T0008	Colorectal Tumor
T0010	Human Infant Brain
T0023	Human Pancreatic Carcinoma
T0039	HSA 172 Cells
T0040	HSC172 cells
T0041	Jurkat T-cell G1 phase
T0042	Jurkat T-Cell, S phase
T0048	Human Aortic Endothelium
T0049	Aorta endothelial cells + TNF-a
T0060	Human White Adipose
T0067	Human Thyroid
T0069	Human Uterus, normal
T0082	Human Adult Retina
T0109	Human (HCC) cell line liver (mouse) metastasis, remake
T0110	Human colon carcinoma (HCC) cell line, remake
T0114	Human (Caco-2) cell line, adenocarcinoma, colon, remake

Table 5

OMIM ID	OMIM Description
101000	Malignant mesothelioma, sporadic (3) Meningioma, NF2-related, sporadic (3) Schwannoma, sporadic (3) Neurofibromatosis, type 2 (3) Neurolemmomatosis (3)
123620	Cataract, cerulean, type 2, 601547 (3)
138981	Pulmonary alveolar proteinosis, 265120 (3)
188826	Sorsby fundus dystrophy, 136900 (3)
600850	Schizophrenia disorder-4 (2)
601669	Hirschsprung disease, one form (2) (?)

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification , such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the secreted protein.

The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or a cDNA contained in ATCC deposit Z. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide encoded by the cDNA contained in ATCC deposit Z. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide sequence encoded by the cDNA contained in ATCC deposit Z are also encompassed by the invention.

Signal Sequences

The present invention also encompasses mature forms of the polypeptide having the polypeptide sequence of SEQ ID NO:Y and/or the polypeptide sequence encoded by the cDNA in a deposited clone. Polynucleotides encoding the mature forms (such as, for example, the polynucleotide sequence in SEQ ID NO:X and/or the

polynucleotide sequence contained in the cDNA of a deposited clone) are also encompassed by the invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretary leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, Virus Res. 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, Nucleic Acids Res. 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, supra.) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called SignalP (Henrik Nielsen et al., Protein Engineering 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis of the amino acid sequences of the secreted proteins described herein by this program provided the results shown in Table 1.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence

shown in SEQ ID NO:Y which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein produced by expression of the polynucleotide sequence of SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone, in a mammalian cell (e.g., COS cells, as desribed below). These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Polynucleotide and Polypeptide Variants

The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X, the complementary strand thereto, and/or the cDNA sequence contained in a deposited clone.

The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:Y and/or encoded by a deposited clone.

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for example, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the nucleotide coding sequence contained in a deposited cDNA clone or the

complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited clone, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

The present invention is also directed to polypeptides which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, for example, the polypeptide sequence shown in SEQ ID NO:Y, the polypeptide sequence encoded by the cDNA contained in a deposited clone, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein).

By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown inTable 1, the ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the presence invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp.

App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identiy are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the lenght of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the

deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, an amino acid sequences shown in Table 1 (SEQ ID NO:Y) or to the amino acid sequence encoded by cDNA contained in a deposited clone can be determined conventionally using known computer programs. A preferred method for determing the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window

Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not

matched/aligned with the query sequnce are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as E. coli).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over

3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340

(1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of the present invention having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of the present invention, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of the present invention or fragments thereof (e.g., the mature form and/or other fragments described herein), is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

Polynucleotide and Polypeptide Fragments

The present invention is also directed to polynucleotide fragments of the polynucleotides of the invention.

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of that contained in a deposited clone, or encoding the polypeptide encoded by the cDNA in a deposited clone; is a portion of that shown in SEQ ID NO:X or the complementary strand thereto, or is a portion of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:Y. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:X. In this context "about" includes the particularly recited value, a value larger

or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, or 2001 to the end of SEQ ID NO:X, or the complementary strand thereto, or the cDNA contained in a deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or

smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotides encoding these domains are also contemplated.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a functional activity. By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) polypeptide of invention protein. Such functional activities include, but are not

limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide of the invention for binding) to an antibody to the polypeptide of the invention], immunogenicity (ability to generate antibody which binds to a polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention.

The functional activity of polypeptides of the invention, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the invention for binding to an antibody of the polypeptide of the invention, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where a ligand for a polypeptide of the invention identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., 1995, Microbiol. Rev. 59:94-123. In another embodiment, physiological correlates of binding of a polypeptide of the invention to its substrates (signal transduction) can be assayed.

In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the invention and fragments, variants derivatives and analogs thereof to elicit related biological activity related to that of the polypeptide of the invention (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope of the invention.

Epitopes and Antibodies

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID NO:Y, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC deposit No. Z or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:X or contained in ATCC deposit No. Z under stringent hybridization conditions or lower stringency hybridization conditions as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower stringency hybridization conditions defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays

described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids

have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., supra; Wilson et al., supra, and Bittle et al., J. Gen. Virol., 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 μg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains

of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion desulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni2+ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by

DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region,

CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described

herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6}M, 5×10^{-7} M, 10^{7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, $^{10-12}$ M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferrably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The

invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998);

Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of- interest can be produced by various procedures well known in the art. For example, a polypeptide of

the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples (e.g., Example 16). In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a

polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809;

WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with

the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce

chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its

ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

Polynucleotides Encoding Antibodies

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR

may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are

described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus

expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as Escherichia coli, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., Gene 45:101 (1986); Cockett et al., Bio/Technology 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non- essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript,

glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217

(1993); May, 1993, TIB TECH 11(5):155-215); and hygro, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography),

centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or

conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in their entireties).

As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide- linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the

tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include 125I, 131I, 111In or 99Tc.

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, 213Bi. A cytotoxin

or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, a-interferon, ß-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al., Int. Immunol., 6*:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti- angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports

include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Immunophenotyping

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with

antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al., Cell, 96*:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the

art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., 32P or 125I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the

coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., 3H or 125I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., 3H or 125I) in the presence of increasing amounts of an unlabeled second antibody.

Therapeutic Uses

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or

conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or Kd less than $5 X 10^{-2}$ M, 10^{-2} M, $5 X 10^{-3}$ M, 10^{-3} M, $5 X 10^{-4}$ M, 10^{-4} M, $5 X 10^{-5}$ M, 10^{-5} M, $5 X 10^{-6}$ M, 10^{-6} M, $5 X 10^{-7}$ M, 10^{-7} M, $5 X 10^{-8}$ M,

10^{-8} M, 5 X 10^{-9} M, 10^{-9} M, 5 X 10^{-10} M, 10^{-10} M, 5 X 10^{-11} M, 10^{-11} M, 5 X 10^{-12} M, 10^{-12} M, 5 X 10^{-13} M, 10^{-13} M, 5 X 10^{-14} M, 10^{-14} M, 5 X 10^{-15} M, and 10^{-15} M.

Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue- specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA

86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143- 155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Cline, Pharmac. Ther. 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. Demonstration of Therapeutic or Prophylactic Activity

The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Therapeutic/Prophylactic Administration and Composition

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical

composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material,

including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J.Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g.,

Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. . Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human

antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. Diagnosis and Imaging

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of

actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of

99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patent using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

<u>Kits</u>

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of

the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface- bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

Fusion Proteins

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the

polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).)

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a

fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the

vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells (e.g., Saccharomyces cerevisiae or Pichia pastoris (ATCC Accession No. 201178)); insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ,pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express the polypeptide of the present invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A

main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O_2. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O_2. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. *See*, Ellis, S.B., *et al., Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J, *et al., Yeast* 5:167-77 (1989); Tschopp, J.F., *et al., Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a protein of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an

expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with the polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination, resulting in the formation of a new transcription unit (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; U.S. Patent No. 5,733,761, issued March 31, 1998; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide sequence of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino

acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptides which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent NO: 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used,

depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions,

substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

The polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, *Therapeutics*) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone (including fragments, variants, splice variants, and fusion proteins, corresponding to these polypeptides as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (*e.g.*, containing polypeptides having identical or different amino acid sequences) or a homotrimer (*e.g.*, containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (*i.e.*, polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example,

liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in the sequence listing, or contained in the polypeptide encoded by a deposited clone). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein of the invention.

In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in an Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, oseteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide seuqence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hyrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present invention can be used as a chromosome marker.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are

more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library) .) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the present invention in cells or body fluid from an individual and comparing the measured gene expression level with a standard level of polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder.

In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the present invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the present invention, where each probe has one strand containing a 31'mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a disorder, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the present invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of polynucleotide of the present invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the present invention or the level of the mRNA encoding the polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains the polypeptide of the present invention or mRNA. As indicated, biological samples include body fluids (such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and other tissue

sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferrably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the present invention attached may be used to identify polymorphisms between the polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, including cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, Science 254, 1497 (1991); and M. Egholm, O. Buchardt, L.Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, Nature 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform

multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point (T.sub.m) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

The present invention is useful for detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative diseases, disorders, and/or conditions are often associated with inappropriate activation of proto-oncogenes. (Gelmann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in Neoplastic Diseases of the Blood, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Gelmann et al., supra) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Gelmann et al., supra) Indeed, the human counterparts of the oncogenes involved in some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Gelmann et al., supra)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO

91/15580) However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness would not be limited to treatment of proliferative diseases, disorders, and/or conditions of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression,CRCPress, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat or prevent disease.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the

present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant,urine,fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell . Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99mTc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-

radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, 131I, 112In, 99mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Moreover, polypeptides of the present invention can be used to treat, prevent, and/or diagnose disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor supressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat, prevent, and/or diagnose disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

Gene Therapy Methods

Another aspect of the present invention is to gene therapy methods for treatingor preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of a polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the invention that operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the invention *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, see Belldegrun et al., J. Natl. Cancer Inst., 85:207-216 (1993); Ferrantini et al., Cancer Research, 53:107-1112 (1993); Ferrantini et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura et al., Cancer Research 50: 5102-5106 (1990); Santodonato, et al., Human Gene Therapy 7:1-10 (1996); Santodonato, et al., Gene Therapy 4:1246-1255 (1997); and Zhang, et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations,

lipofectin or precipitating agents and the like. However, the polynucleotides of the invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs of the invention used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of polynucleotide sequence of the invention. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotides of the invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct of the invention can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular,

fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked*nucleic* acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs of the invention are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA , 86:6077-6081 (1989), which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem., 265:10189-10192 (1990), which is herein incorporated by reference), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication NO: WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., Felgner et al., Proc. Natl. Acad. Sci. USA, 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP

starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., Methods of Immunology , 101:512-527 (1983), which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include Ca^{2+}-EDTA chelation (Papahadjopoulos et al., Biochim. Biophys. Acta,

394:483 (1975); Wilson et al., Cell , 17:77 (1979)); ether injection (Deamer et al., Biochim. Biophys. Acta, 443:629 (1976); Ostro et al., Biochem. Biophys. Res. Commun., 76:836 (1977); Fraley et al., Proc. Natl. Acad. Sci. USA, 76:3348 (1979)); detergent dialysis (Enoch et al., Proc. Natl. Acad. Sci. USA , 76:145 (1979)); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem., 255:10431 (1980); Szoka et al., Proc. Natl. Acad. Sci. USA , 75:145 (1978); Schaefer-Ridder et al., Science, 215:166 (1982)), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent NO: 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

In certain embodiments, cells are engineered, *ex vivo* or *in vivo*, using a retroviral particle containing RNA which comprises a sequence encoding polypeptides of the invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy , 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any

means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding polypeptides of the invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express polypeptides of the invention.

In certain other embodiments, cells are engineered, *ex vivo* or *in vivo*, with polynucleotides of the invention contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses polypeptides of the invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartzet al., Am. Rev. Respir. Dis., 109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld et al.,Science , 252:431-434 (1991); Rosenfeld et al., Cell, 68:143-155 (1992)). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green et al. Proc. Natl. Acad. Sci. USA , 76:6606 (1979)).

Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel., 3:499-503 (1993); Rosenfeld et al., Cell , 68:143-155 (1992); Engelhardt et al., Human Genet. Ther., 4:759-769 (1993); Yang et al., Nature Genet., 7:362-369 (1994); Wilson et al., Nature , 365:691-692 (1993); and U.S. Patent NO: 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the El region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other

varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, *ex vivo* or *in vivo*, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, Curr. Topics in Microbiol. Immunol., 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct containing polynucleotides of the invention is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct of the invention. These viral particles are then used to transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express the desired gene product.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding the polypeptide sequence of interest) via homologous recombination (see, e.g., U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al., Nature, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5′ end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5′ and 3′ ends. Preferably, the 3′ end of the first targeting sequence contains the same restriction enzyme site as the 5′ end of the amplified promoter and the 5′ end of the second targeting sequence contains the same restriction site as the 3′ end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

The polynucleotides encoding polypeptides of the present invention may be administered along with other polynucleotides encoding other angiongenic proteins. Angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2 (VEGF-C), VEGF-3 (VEGF-B), epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

Preferably, the polynucleotide encoding a polypeptide of the invention contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5′ end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers. (Kaneda et al., Science, 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is

administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA , 189:11277-11281 (1992), which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian. Therapeutic compositions of

the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly

Biological Activities

The polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides or polypeptides, or agonists or antagonists could be used to treat the associated disease.

Immune Activity

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of the immune system, by, for example, activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of hematopoietic cells. Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a decrease in certain (or many) types hematopoietic cells.

Examples of immunologic deficiency syndromes include, but are not limited to: blood protein diseases, disorders, and/or conditions (e.g., agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, polynucleotides or polypeptides, and/or agonists or antagonists of the present invention could be used to treat or prevent blood coagulation diseases, disorders, and/or conditions (e.g., afibrinogenemia, factor deficiencies), blood platelet diseases, disorders, and/or conditions (e.g., thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment or prevention of heart attacks (infarction), strokes, or scarring.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of polynucleotides and polypeptides of the invention that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Autoimmune diseases or disorders that may be treated, prevented, and/or diagnosed by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, one or more of the following:

autoimmune hemolytic anemia, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, autoimmunocytopenia, hemolytic anemia, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (e.g, IgA nephropathy), Multiple Sclerosis, Neuritis, Uveitis Ophthalmia, Polyendocrinopathies, Purpura (e.g., Henloch-Scoenlein purpura), Reiter's Disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, Autism, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye, autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis, systemic lupus erhythematosus, Goodpasture's syndrome, Pemphigus, Receptor autoimmunities such as, for example, (a) Graves' Disease, (b) Myasthenia Gravis, and (c) insulin resistance, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, rheumatoid arthritis, schleroderma with anti-collagen antibodies, mixed connective tissue disease, polymyositis/dermatomyositis, pernicious anemia, idiopathic Addison's disease, infertility, glomerulonephritis such as primary glomerulonephritis and IgA nephropathy, bullous pemphigoid, Sjogren's syndrome, diabetes millitus, and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis), chronic active hepatitis, primary biliary cirrhosis, other endocrine gland failure, vitiligo, vasculitis, post-MI, cardiotomy syndrome, urticaria, atopic dermatitis, asthma, inflammatory myopathies, and other inflammatory, granulamatous, degenerative, and atrophic disorders.

Additional autoimmune disorders (that are probable) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, rheumatoid arthritis (often characterized, e.g., by immune complexes in joints), scleroderma with anti-collagen antibodies (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed connective tissue disease (often characterized, e.g., by antibodies to extractable nuclear antigens (e.g., ribonucleoprotein)), polymyositis (often characterized, e.g., by nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell, microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility (often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often characterized, e.g., by

glomerular basement membrane antibodies or immune complexes), bullous pemphigoid (often characterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes millitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

Additional autoimmune disorders (that are possible) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, chronic active hepatitis (often characterized, e.g., by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitchondrial antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by myocardial antibodies), cardiotomy syndrome (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), and many other inflammatory, granulamatous, degenerative, and atrophic disorders.

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, and/or diagnosed using for example, antagonists or agonists, polypeptides or polynucleotides, or antibodies of the present invention.

In a preferred embodiment polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among B cell and/or T cell immunodeficient individuals.

B cell immunodeficiencies that may be ameliorated or treated by administering the polypeptides or polynucleotides of the invention, and/or agonists thereof, include, but are not limited to, severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-linked agammaglobulinemia (XLA), Bruton's disease, congenital

agammaglobulinemia, X-linked infantile agammaglobulinemia, acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspecified hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency (CVI) (acquired), Wiskott-Aldrich Syndrome (WAS), X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated Igs, immunodeficiency with thymoma, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe congenital leukopenia, thymic alymophoplasia-aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome-combined immunodeficiency with Igs, purine nucleoside phosphorylase deficiency (PNP), MHC Class II deficiency (Bare Lymphocyte Syndrome) and severe combined immunodeficiency.

T cell deficiencies that may be ameliorated or treated by administering the polypeptides or polynucleotides of the invention, and/or agonists thereof include, but are not limited to, for example, DiGeorge anomaly, thymic hypoplasia, third and fourth pharyngeal pouch syndrome, 22q11.2 deletion, chronic mucocutaneous candidiasis, natural killer cell deficiency (NK), idiopathic CD4+ T-lymphocytopenia, immunodeficiency with predominant T cell defect (unspecified), and unspecified immunodeficiency of cell mediated immunity. In specific embodiments, DiGeorge anomaly or conditions associated with DiGeorge anomaly are ameliorated or treated by, for example, administering the polypeptides or polynucleotides of the invention, or antagonists or agonists thereof.

Other immunodeficiencies that may be ameliorated or treated by administering polypeptides or polynucleotides of the invention, and/or agonists thereof, include, but are not limited to, severe combined immunodeficiency (SCID; e.g., X-linked SCID, autosomal SCID, and adenosine deaminase deficiency), ataxia-telangiectasia, Wiskott-Aldrich syndrome, short-limber dwarfism, X-linked lymphoproliferative

syndrome (XLP), Nezelof syndrome (e.g., purine nucleoside phosphorylase deficiency), MHC Class II deficiency. In specific embodiments, ataxia-telangiectasia or conditions associated with ataxia-telangiectasia are ameliorated or treated by administering the polypeptides or polynucleotides of the invention, and/or agonists thereof.

In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment, systemic lupus erythemosus is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment, idiopathic thrombocytopenia purpura is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment IgA nephropathy is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, and/or diagnosed using antibodies against the protein of the invention.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, and/or diagnosed using polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof. Moreover, these molecules can be used to treat, prevent, and/or diagnose anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

Moreover, inflammatory conditions may also be treated, diagnosed, and/or prevented with polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. Such inflammatory conditions include, but are not limited to, for example, respiratory disorders (such as, e.g., asthma and allergy); gastrointestinal disorders (such as, e.g., inflammatory bowel disease); cancers (such as, e.g., gastric, ovarian, lung, bladder, liver, and breast); CNS disorders (such as, e.g., multiple sclerosis, blood-brain barrier permeability, ischemic brain injury and/or

stroke, traumatic brain injury, neurodegenerative disorders (such as, e.g., Parkinson's disease and Alzheimer's disease), AIDS-related dementia, and prion disease); cardiovascular disorders (such as, e.g., atherosclerosis, myocarditis, cardiovascular disease, and cardiopulmonary bypass complications); as well as many additional diseases, conditions, and disorders that are characterized by inflammation (such as, e.g., chronic hepatitis (B and C), rheumatoid arthritis, gout, trauma, septic shock, pancreatitis, sarcoidosis, dermatitis, renal ischemia-reperfusion injury, Grave's disease, systemic lupus erythematosis, diabetes mellitus (i.e., type 1 diabetes), and allogenic transplant rejection).

In specific embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, are useful to treat, diagnose, and/or prevent transplantation rejections, graft-versus-host disease, autoimmune and inflammatory diseases (e.g., immune complex-induced vasculitis, glomerulonephritis, hemolytic anemia, myasthenia gravis, type II collagen-induced arthritis, experimental allergic and hyperacute xenograft rejection, rheumatoid arthritis, and systemic lupus erythematosus (SLE). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. Polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may also be used to modulate and/or diagnose inflammation. For example, since polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists of the invention may inhibit the activation, proliferation and/or differentiation of cells involved in an inflammatory response, these molecules can be used to treat, diagnose, or prognose, inflammatory conditions, both chronic and acute conditions, including, but not limited to, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced

lung injury, inflammatory bowel disease, Crohn's disease, and resulting from over production of cytokines (e.g., TNF or IL-1.).

Polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the invention can be used to treat, detect, and/or prevent infectious agents. For example, by increasing the immune response, particularly increasing the proliferation activation and/or differentiation of B and/or T cells, infectious diseases may be treated, detected, and/or prevented. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may also directly inhibit the infectious agent (refer to section of application listing infectious agents, etc), without necessarily eliciting an immune response.

Additional preferred embodiments of the invention include, but are not limited to, the use of polypeptides, antibodies, polynucleotides and/or agonists or antagonists in the following applications:

Administration to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to induce higher affinity antibody production (e.g., IgG, IgA, IgM, and IgE), and/or to increase an immune response.

Administration to an animal (including, but not limited to, those listed above, and also including transgenic animals) incapable of producing functional endogenous antibody molecules or having an otherwise compromised endogenous immune system, but which is capable of producing human immunoglobulin molecules by means of a reconstituted or partially reconstituted immune system from another animal (see, e.g., published PCT Application Nos. WO98/24893, WO/9634096, WO/9633735, and WO/9110741.

A vaccine adjuvant that enhances immune responsiveness to specific antigen.

An adjuvant to enhance tumor-specific immune responses.

An adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an

adjuvant, include virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, Respiratory syncytial virus, Dengue, Rotavirus, Japanese B encephalitis, Influenza A and B, Parainfluenza, Measles, Cytomegalovirus, Rabies, Junin, Chikungunya, Rift Valley fever, Herpes simplex, and yellow fever.

An adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, Diphtheria, botulism, and meningitis type B. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: *Vibrio cholerae, Mycobacterium leprae, Salmonella typhi, Salmonella paratyphi, Meisseria meningitidis, Streptococcus pneumoniae*, Group B streptococcus, *Shigella spp.*, Enterotoxigenic *Escherichia coli*, Enterohemorrhagic *E. coli*, *Borrelia burgdorferi*, and Plasmodium (malaria).

An adjuvant to enhance anti-parasitic immune responses. Anti-parasitic immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a parasite. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to Plasmodium (malaria).

As a stimulator of B cell responsiveness to pathogens.

As an activator of T cells.

As an agent that elevates the immune status of an individual prior to their receipt of immunosuppressive therapies.

As an agent to induce higher affinity antibodies.

As an agent to increase serum immunoglobulin concentrations.

As an agent to accelerate recovery of immunocompromised individuals.

As an agent to boost immunoresponsiveness among aged populations.

As an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogeneic or xenogeneic organ transplantation). With respect to transplantation, compositions of the invention may be administered prior to, concomitant with, and/or after transplantation. In a specific embodiment, compositions of the invention are administered after transplantation, prior to the beginning of recovery of T-cell populations. In another specific embodiment, compositions of the invention are first administered after transplantation after the beginning of recovery of T cell populations, but prior to full recovery of B cell populations.

As an agent to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell function that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are not limited to, HIV Infection, AIDS, bone marrow transplant, and B cell chronic lymphocytic leukemia (CLL).

As an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, recovery from surgery.

As a regulator of antigen presentation by monocytes, dendritic cells, and/or B-cells. In one embodiment, polynucleotides, polypeptides, antibodies, and/or

agonists or antagonists of the present invention enhance antigen presentation or antagonizes antigen presentation in vitro or in vivo. Moreover, in related embodiments, said enhancement or antagonization of antigen presentation may be useful as an anti-tumor treatment or to modulate the immune system.

As an agent to direct an individuals immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

As a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

As a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodificiency.

As a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect.

As a gene-based therapy for genetically inherited disorders resulting in immuno-incompetence such as observed among SCID patients.

As an antigen for the generation of antibodies to inhibit or enhance immune mediated responses against polypeptides of the invention.

As a means of activating T cells.

As a means of activating monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leshmania.

As pretreatment of bone marrow samples prior to transplant. Such treatment would increase B cell representation and thus accelerate recover.

As a means of regulating secreted cytokines that are elicited by polypeptides of the invention.

Additionally, polypeptides or polynucleotides of the invention, and/or agonists thereof, may be used to treat or prevent IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema.

All of the above described applications as they may apply to veterinary medicine.

Antagonists of the invention include, for example, binding and/or inhibitory antibodies, antisense nucleic acids, or ribozymes. These would be expected to reverse many of the activities of the ligand described above as well as find clinical or practical application as:

A means of blocking various aspects of immune responses to foreign agents or self. Examples include autoimmune disorders such as lupus, and arthritis, as well as immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and pathogens.

A therapy for preventing the B cell proliferation and Ig secretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythramatosus and MS.

An inhibitor of B and/or T cell migration in endothelial cells. This activity disrupts tissue architecture or cognate responses and is useful, for example in disrupting immune responses, and blocking sepsis.

An inhibitor of graft versus host disease or transplant rejection.

A therapy for B cell and/or T cell malignancies such as ALL, Hodgkins disease, non-Hodgkins lymphoma, Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, and EBV-transformed diseases.

A therapy for chronic hypergammaglobulinemeia evident in such diseases as monoclonalgammopathy of undetermined significance (MGUS), Waldenstrom's disease, related idiopathic monoclonalgammopathies, and plasmacytomas.

A therapy for decreasing cellular proliferation of Large B-cell Lymphomas.

A means of decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

An immunosuppressive agent(s).

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate IgE concentrations in vitro or in vivo.

In another embodiment, administration of polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the invention, may be used to treat or prevent IgE-mediated allergic reactions including, but not limited to, asthma, rhinitis, and eczema.

The agonists and antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

The agonists or antagonists may be employed for instance to inhibit polypeptide chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain auto-immune and chronic inflammatory and infective diseases. Examples of autoimmune diseases are described herein and include multiple sclerosis, and insulin-dependent diabetes. The antagonists or agonists may also be employed to treat infectious diseases including silicosis, sarcoidosis, idiopathic pulmonary fibrosis by, for example, preventing the recruitment and activation of mononuclear phagocytes. They may also be employed to treat idiopathic hyper-eosinophilic syndrome by, for example, preventing eosinophil production and migration. The antagonists or agonists or may also be employed for treating atherosclerosis, for example, by preventing monocyte infiltration in the artery wall.

Antibodies against polypeptides of the invention may be employed to treat ARDS.

Agonists and/or antagonists of the invention also have uses in stimulating wound and tissue repair, stimulating angiogenesis, stimulating the repair of vascular or lymphatic diseases or disorders. Additionally, agonists and antagonists of the invention may be used to stimulate the regeneration of mucosal surfaces.

In a specific embodiment, polynucleotides or polypeptides, and/or agonists thereof are used to treat or prevent a disorder characterized by primary or acquired immunodeficiency, deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, polynucleotides or polypeptides, and/or agonists thereof may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL,

recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carnii.

In another embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention are used to treat, and/or diagnose an individual having common variable immunodeficiency disease ("CVID"; also known as "acquired agammaglobulinemia" and "acquired hypogammaglobulinemia") or a subset of this disease.

In a specific embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to treat, diagnose, and/or prevent (1) cancers or neoplasms and (2) autoimmune cell or tissue-related cancers or neoplasms. In a preferred embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat, diagnose, and/or prevent acute myelogeneous leukemia. In a further preferred embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat, diagnose, and/or prevent, chronic myelogeneous leukemia, multiple myeloma, non-Hodgkins lymphoma, and/or Hodgkins disease.

In another specific embodiment, polynucleotides or polypeptides, and/or agonists or antagonists of the invention may be used to treat, diagnose, prognose, and/or prevent selective IgA deficiency, myeloperoxidase deficiency, C2 deficiency, ataxia-telangiectasia, DiGeorge anomaly, common variable immunodeficiency (CVI), X-linked agammaglobulinemia, severe combined immunodeficiency (SCID), chronic granulomatous disease (CGD), and Wiskott-Aldrich syndrome.

Examples of autoimmune disorders that can be treated or detected are described above and also include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary

Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye disease.

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prognosed, prevented, and/or diagnosed using antibodies against the polypeptide of the invention.

As an agent to boost immunoresponsiveness among B cell immunodeficient individuals, such as, for example, an individual who has undergone a partial or complete splenectomy.

Additionally, polynucleotides, polypeptides, and/or antagonists of the invention may affect apoptosis, and therefore, would be useful in treating a number of diseases associated with increased cell survival or the inhibition of apoptosis. For example, diseases associated with increased cell survival or the inhibition of apoptosis that could be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metastisis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g.,

acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestosis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Hyperproliferative diseases and/or disorders that could be detected and/or treated by polynucleotides, polypeptides, and/or antagonists of the invention, include, but are not limited to neoplasms located in the: liver, abdomen, bone, breast, digestive system, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstron's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

Hyperproliferative Disorders

A polynucleotides or polypeptides, or agonists or antagonists of the invention can be used to treat, prevent, and/or diagnose hyperproliferative diseases, disorders, and/or conditions, including neoplasms. A polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, a polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative diseases, disorders, and/or conditions can be treated, prevented, and/or diagnosed. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating, preventing, and/or diagnosing hyperproliferative diseases, disorders, and/or conditions, such as a chemotherapeutic agent.

Examples of hyperproliferative diseases, disorders, and/or conditions that can be treated, prevented, and/or diagnosed by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative diseases, disorders, and/or conditions can also be treated, prevented, and/or diagnosed by a polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative diseases, disorders, and/or conditions include, but are not limited to: hypergammaglobulinemia, lymphoproliferative diseases, disorders, and/or conditions, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstron's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

One preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

Thus, the present invention provides a method for treating or preventing cell proliferative diseases, disorders, and/or conditions by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating or preventing cell-proliferative diseases, disorders, and/or conditions in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the poynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferrably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference).

In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and

the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating, preventing, and/or diagnosing one or more of the described diseases, disorders, and/or conditions. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

In particular, the antibodies, fragments and derivatives of the present invention are useful for treating, preventing, and/or diagnosing a subject having or developing cell proliferative and/or differentiation diseases, disorders, and/or conditions as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors, for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of diseases, disorders, and/or conditions related to polynucleotides or polypeptides, including fragements thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragements thereof. Preferred binding affinities include those with a dissociation constant or Kd less than $5X10^{-6}M$, $10^{-6}M$, $5X10^{-7}M$, $10^{-7}M$, $5X10^{-8}M$, $10^{-8}M$, $5X10^{-9}M$, $10^{-9}M$, $5X10^{-10}M$, $10^{-10}M$, $5X10^{-11}M$, $10^{-11}M$, $5X10^{-12}M$, $10^{-12}M$, $5X10^{-13}M$, $10^{-13}M$, $5X10^{-14}M$, $10^{-14}M$, $5X10^{-15}M$, and $10^{-15}M$.

Moreover, polypeptides of the present invention are useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-

specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al., Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs or adjuviants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins (See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by reference).

Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such thereapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing polypeptides or polypeptide antibodes associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodes of the invention may be associated with with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

Cardiovascular Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the invention may be used to treat, prevent, and/or diagnose cardiovascular diseases, disorders, and/or conditions, including peripheral artery disease, such as limb ischemia.

Cardiovascular diseases, disorders, and/or conditions include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogy of Fallot, ventricular heart septal defects.

Cardiovascular diseases, disorders, and/or conditions also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac

arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

Heart valve disease include aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms, angiodysplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular diseases, disorders, and/or conditions, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

Cerebrovascular diseases, disorders, and/or conditions include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subaraxhnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromoboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polynucleotides or polypeptides, or agonists or antagonists of the invention, are especially effective for the treatment of critical limb ischemia and coronary disease.

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppositorial solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides of the invention may be administered as part of a *Therapeutic*, described in more detail below. Methods of delivering polynucleotides of the invention are described in more detail herein.

Anti-Angiogenesis Activity

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell* *56*:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye diseases, disorders, and/or conditions, and psoriasis. See, e.g., reviews by Moses *et al.*,

Biotech. *9*:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, *333*:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* *29*:401-411 (1985); Folkman, Advances in Cancer Research, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Opthalmol.* *94*:715-743 (1982); and Folkman *et al.*, Science *221*:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* *235*:442-447 (1987).

The present invention provides for treatment of diseases, disorders, and/or conditions associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*, Medicine, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)).Thus, the present invention provides a method of treating, preventing, and/or diagnosing an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists may be utilized in a variety of additional methods in order to therapeutically treator prevent a cancer or tumor. Cancers which may be treated, prevented, and/or diagnosed with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non- small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, in order to treat or prevent cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.

Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating, preventing, and/or diagnosing other diseases, disorders, and/or conditions, besides cancers, which involve angiogenesis. These diseases, disorders, and/or conditions include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; artheroscleric plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uvietis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating, preventing, and/or diagnosing hypertrophic scars and keloids, comprising the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days

after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating, preventing, and/or diagnosing neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

Moreover, Ocular diseases, disorders, and/or conditions associated with neovascularization which can be treated, prevented, and/or diagnosed with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al., Am. J. Ophthal. 85*:704-710 (1978) and Gartner *et al., Surv. Ophthal. 22*:291-312 (1978).

Thus, within one aspect of the present invention methods are provided for treating or preventing neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however, capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of diseases, disorders, and/or conditions can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and

antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbic corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for treating or preventing neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat or prevent early forms of neovascular glaucoma. Within other

embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating or preventing proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating or preventing retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreous injection and/or via intraocular implants.

Additionally, diseases, disorders, and/or conditions which can be treated, prevented, and/or diagnosed with the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, diseases, disorders, and/or conditions and/or states, which can be treated, prevented, and/or diagnosed with the the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft

rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uvietis, delayed wound healing, endometriosis, vascluogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophiliac joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochele minalia quintosa), ulcers (Helicobacter pylori), Bartonellosis and bacillary angiomatosis.

In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or agonists may also be used in controlling menstruation or administered as either a peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti- angiogenic compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the

invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST";

Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaminolmidazole; and metalloproteinase inhibitors such as BB94.

Diseases at the Cellular Level

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated, prevented, and/or diagnosed by the polynucleotides or polypeptides and/or antagonists or agonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated, prevented or diagnosed by the polynucleotides or polypeptides, or agonists or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic)

leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated, prevented, and/or diagnosed by the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, include AIDS; neurodegenerative diseases, disorders, and/or conditions (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestosis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Wound Healing and Epithelial Cell Proliferation

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associted with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote dermal reestablishment subsequent to dermal loss

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are a non-exhaustive list of grafts that polynucleotides or polypeptides, agonists or antagonists of the invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepdermic grafts, avacular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omenpal graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, will also produce changes in hepatocyte proliferation,

and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intesting, and large intestine. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may have a cytoprotective effect on the small intestine mucosa. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could also be used to treat gastric and doudenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflamamatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel

disease. Treatment with the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat diseases associate with the under expression of the polynucleotides of the invention.

Moreover, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to prevent and heal damage to the lungs due to various pathological states. A growth factor such as the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and brochiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of aveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated, prevented, and/or diagnosed using the polynucleotides or polypeptides, and/or agonists or antagonists of the invention. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary displasia, in premature infants.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetraholoride and other hepatotoxins known in the art).

In addition, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, the polynucleotides or polypeptides, and/or agonists or antagonists of the

invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

Neurological Diseases

Nervous system diseases, disorders, and/or conditions, which can be treated, prevented, and/or diagnosed with the compositions of the invention (e.g., polypeptides, polynucleotides, and/or agonists or antagonists), include, but are not limited to, nervous system injuries, and diseases, disorders, and/or conditions which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated, prevented, and/or diagnosed in a patient (including human and non-human mammalian patients) according to the invention, include but are not limited to, the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems: (1) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis; (5) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis (ALS); (6) lesions associated with nutritional diseases, disorders, and/or conditions, in which a

portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including, but not limited to, multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

In a preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects of cerebral hypoxia. According to this embodiment, the compositions of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral hypoxia. In one aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral ischemia. In another aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral infarction. In another aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose or prevent neural cell injury associated with a stroke. In a further aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with a heart attack.

The compositions of the invention which are useful for treating or preventing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, compositions of the invention which elicit any of the following effects may be useful according to the invention: (1) increased survival time of neurons in culture;

(2) increased sprouting of neurons in culture or *in vivo*; (3) increased production of a neuron-associated molecule in culture or *in vivo*, *e.g.*, choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron dysfunction *in vivo*. Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, the method set forth in Arakawa et al. (J. Neurosci. 10:3507-3515 (1990)); increased sprouting of neurons may be detected by methods known in the art, such as, for example, the methods set forth in Pestronk et al. (Exp. Neurol. 70:65-82 (1980)) or Brown et al. (Ann. Rev. Neurosci. 4:17-42 (1981)); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron diseases, disorders, and/or conditions that may be treated, prevented, and/or diagnosed according to the invention include, but are not limited to, diseases, disorders, and/or conditions such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as diseases, disorders, and/or conditions that selectively affect neurons such as amyotrophic lateral sclerosis, and including, but not limited to, progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

Infectious Disease

A polypeptide or polynucleotide and/or agonist or antagonist of the present invention can be used to treat, prevent, and/or diagnose infectious agents. For example, by increasing the immune response, particularly increasing the proliferation

and differentiation of B and/or T cells, infectious diseases may be treated, prevented, and/or diagnosed. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polypeptide or polynucleotide and/or agonist or antagonist of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiollitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention

are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose AIDS.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Cryptococcus neoformans, Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia (e.g., Borrelia burgdorferi), Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, E. coli (e.g., Enterotoxigenic E. coli and Enterohemorrhagic E. coli), Enterobacteriaceae (Klebsiella, Salmonella (e.g., Salmonella typhi, and Salmonella paratyphi), Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Mycobacterium leprae, Vibrio cholerae, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Menigococcal), Meisseria meningitidis, Pasteurellacea Infections (e.g., Actinobacillus, Heamophilus (e.g., Heamophilus influenza type B), Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, Shigella spp., Staphylococcal, Meningiococcal, Pneumococcal and Streptococcal (e.g., Streptococcus pneumoniae and Group B Streptococcus). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., mengitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections.

Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: tetanus, Diptheria, botulism, and/or meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparium, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used totreat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose malaria.

Preferably, treatment or prevention using a polypeptide or polynucleotide and/or agonist or antagonist of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

Regeneration

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues

could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteocarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. A polynucleotide or polypeptide and/or agonist or antagonist of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated, prevented, and/or diagnosed include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using a polynucleotide or polypeptide and/or agonist or antagonist of the present invention to proliferate and differentiate nerve cells. Diseases that could be treated, prevented, and/or diagnosed using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic diseases, disorders, and/or conditions (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stoke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated, prevented, and/or diagnosed using the polynucleotide or polypeptide and/or agonist or antagonist of the present invention.

<u>**Chemotaxis**</u>

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may have chemotaxis activity. A chemotaxic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase chemotaxic activity of particular cells. These chemotactic molecules can then be used to treat, prevent, and/or diagnose inflammation, infection, hyperproliferative diseases, disorders, and/or conditions, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotaxic molecules can be used to treat, prevent, and/or diagnose wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat, prevent, and/or diagnose wounds.

It is also contemplated that a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may inhibit chemotactic activity. These molecules could also be used totreat, prevent, and/or diagnose diseases, disorders, and/or conditions. Thus, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention could be used as an inhibitor of chemotaxis.

Binding Activity

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors),or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology

1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or *E. coli*. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

Additionally, the receptor to which a polypeptide of the invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided

into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labelled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to auto-radiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of polypeptides of the invention thereby effectively generating agonists and antagonists of polypeptides of the invention. See generally, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, S. Trends Biotechnol. 16(2):76-82 (1998); Hansson, L. O., et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. Biotechniques 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and corresponding polypeptides of the invention may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired polynucleotide sequence of the invention molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides of the invention may be altered by being subjected to random mutagenesis by error-prone

PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptides of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptides of the invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and 3[H] thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of 3[H] thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of 3[H] thymidine. Both agonist and antagonist compounds may be identified by this procedure.

In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled

polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat, prevent, and/or diagnose disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from suitably manipulated cells or tissues. Therefore, the invention includes a method of identifying compounds which bind to the polypeptides of the invention comprising the steps of: (a) incubating a candidate binding compound with the polypeptide; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with the polypeptide, (b) assaying a biological activity , and (b) determining if a biological activity of the polypeptide has been altered.

Also, one could identify molecules bind a polypeptide of the invention experimentally by using the beta-pleated sheet regions contained in the polypeptide sequence of the protein. Accordingly, specific embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, the amino acid sequence of each beta pleated sheet regions in a disclosed polypeptide sequence. Additional embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, any combination or all of contained in the polypeptide sequences of the invention. Additional preferred embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, the amino acid sequence of each of the beta pleated sheet regions in one of the polypeptide sequences of the invention. Additional

embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, any combination or all of the beta pleated sheet regions in one of the polypeptide sequences of the invention.

<u>Targeted Delivery</u>

In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed

antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

Drug Screening

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound

form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

Antisense And Ribozyme (Antagonists)

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained a deposited clone. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, Neurochem., 56:560 (1991). Oligodeoxynucleotides as Anitsense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix

formation is discussed in, for instance, Lee et al., Nucleic Acids Research, 6:3073 (1979); Cooney et al., Science, 241:456 (1988); and Dervan et al., Science, 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoR1 site on the 5 end and a HindIII site on the 3 end. Next, the pair of oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl2, 10MM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoR1/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid of the invention. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding a polypeptide of the invention, or fragments thereof, can be by any promoter known in

the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, Nature, 29:304-310 (1981), the promoter contained in the 3′ long terminal repeat of Rous sarcoma virus (Yamamoto et al., Cell, 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., Proc. Natl. Acad. Sci. U.S.A., 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster et al., Nature, 296:39-42 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of interest. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids of the invention, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA sequence of the invention it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5′ end of the message, *e.g.*, the 5′ untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3′ untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., *Nature*, 372:333-335 (1994). Thus, oligonucleotides complementary to either the 5′ - or 3′ - non- translated, non-coding regions of a polynucleotide sequence of the invention could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5′ untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the

5′ -, 3′ - or coding region of mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556 (1989); Lemaitre et al., Proc. Natl. Acad. Sci., 84:648-652 (1987); PCT Publication NO: WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication NO: WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., BioTechniques, 6:958-976 (1988)) or intercalating agents. (See, e.g., Zon, Pharm. Res., 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5′-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v),

5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res., 15:6625-6641 (1987)). The oligonucleotide is a 2-0-methylribonucleotide (Inoue et al., Nucl. Acids Res., 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1987)).

Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res., 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A., 85:7448-7451 (1988)), etc.

While antisense nucleotides complementary to the coding region sequence of the invention could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science, 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs corresponding to the polynucleotides of the invention, the use of hammerhead

ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5′-UG-3′. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature, 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within each nucleotide sequence disclosed in the sequence listing. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5′ end of the mRNA corresponding to the polynucleotides of the invention; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express the polynucleotides of the invention in vivo. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirous in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

The antagonist/agonist may also be employed to treat, prevent, and/or diagnose the diseases described herein.

Thus, the invention provides a method of treating or preventing diseases, disorders, and/or conditions, including but not limited to the diseases, disorders, and/or conditions listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.
invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention

Other Activities

The polypeptide of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. These polypeptide may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

The polypeptide may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

The polypeptide of the present invention may also be employed stimulate neuronal growth and to treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. The polypeptide of the invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

The polypeptide of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

The polypeptide of the invention may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, the polypeptides of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

The polypeptide of the invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues.

The polypeptide of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, polypeptides or polynucleotides and/or agonist or antagonists of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, caricadic rhythms, depression (including depressive diseases, disorders, and/or conditions), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

Other Preferred Embodiments

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5′ Nucleotide of the Clone Sequence and ending with the nucleotide at about the position of the 3′ Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5′ Nucleotide of the Start Codon and ending with the nucleotide at about the position of the 3′ Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Similarly preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5′ Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3′ Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X beginning with the nucleotide at about the position of the 5′ Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about

the position of the 3′ Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which comprises a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the material deposited with the American Type Culture Collection and given the ATCC Deposit Number shown in Table 1 for said cDNA Clone Identifier.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the deposit given the ATCC Deposit Number shown in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of the complete open reading frame sequence encoded by said human cDNA clone.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

Also preferred is a polypeptide, wherein said sequence of contiguous amino acids is included in the amino acid sequence of SEQ ID NO:Y in the range of positions beginning with the residue at about the position of the First Amino Acid of the Secreted Portion and ending with the residue at about the Last Amino Acid of the Open Reading Frame as set forth for SEQ ID NO:Y in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a secreted portion of the secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of

polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino

acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making

an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a secreted portion of a human secreted protein comprising an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y beginning with the residue at the position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y wherein Y is an integer set forth in Table 1 and said position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y is defined in Table 1; and an amino acid sequence of a secreted portion of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a secreted protein activity, which method comprises administering to such an individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to increase the level of said protein activity in said individual.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

In specific embodiments of the invention, for each "Contig ID" listed in the fourth column of Table 6, preferably excluded are one or more polynucleotides comprising, or alternatively consisting of, a nucleotide sequence referenced in the fifth column of Table 6 and described by the general formula of a-b, whereas a and b are uniquely determined for the corresponding SEQ ID NO:X referred to in column 3 of Table 6. Further specific embodiments are directed to polynucleotide sequences excluding one, two, three, four, or more of the specific polynucleotide sequences referred to in the fifth column of Table 6. In no way is this listing meant to encompass all of the sequences which may be excluded by the general formula, it is just a

representative example. All references available through these accessions are hereby incorporated by reference in their entirety.

TABLE 6

Gene No.	cDNA Clone ID	NT SEQ ID NO: X	Contig ID	Public Accession Numbers
2	HMWDB84	64	997408	R23544, AA488816, AA489064, AA872894, AA972544, AA913051, AI383891, AI418155, AI683629, AI798127, AI818208, AI933352, AW008595, AW024683, AW150924
3	HNTEO78	13	898200	W23661, AA031909, AA032010, AA587374, AA909360, AA910436, AI244855, AI283238, AI359648, AI421774, AI219587, AI220016
7	HLQEM64	17	897823	R80991, AA223534, AI206292
9	HOEEK12	19	897860	AA025494, AA025810, AA281207, AA281110, AA583008, AA573525, AA575895, AA766389, AA805511, AA862506, AA886103, AA903796, AA910259, AA247875, AA436439, AA481382, AA670356, AA707020, AA907598, AI090351, AI300932, AI201989, AI474935, AI571768, AI139120, AI625333, AI183990, AI193702, AI216895, AI217445, AI243103, AI669935
12	HFKKS66	22	897819	R27630, AA101419, AA237012, AA251103, AA251668, AA281473, AA404990, AA454923, AA568570, AA577414, AA579585, AA749330, AA766073, AA767545, AA814285, AA887846, AA977236, AA644151, AA723179, AA843499, AA885379, AA918582, AI073442, AI081117, AI091024, AA699331, AI270258, AI270269, AI341384, AI370244, AI198390, AI380887, AI382537, AI400803, AI475113, AI498786, AI142821, AI148458, AI188199, AI655439, AI538105
13	HFVJP07	23	897925	R92968, H67099, H80588, N55428, N55496, N68280, N76801, N80976, AI087961, AI148629
14	HTEAM34	24	898364	AA398805, AA435707, AI015821, AA693501, AI203905
16	HMADJ14	26	1099342	AI268407, AI831182, AW450309
16	HMADJ14	68	889659	AI268407
21	HFKIA71	72	900364	T70514, R25870, R73414, H01285, H19477, H19559, N33086, N44657, W03362, W17078, W95610, W95611, AA062594, AA076613, AA076614, AA464409, AA492284, AA508134, AA548685, AA826756, AA908169, AA922967, F19340, D81646, W30716, N90851, AA642347, AA284973, AA293334, AA401715, F20697, AA476287, AA455525, AA434038, AA434103, AA776502, AA779561, D20196, AI284573, AI382533, AI554787, AI479221, AI480421, AI184356, AI203707, AI266745, AI598276,

				AI337934, AI350693, AI589151
22	HOSNU69	32	898152	AA558031, AA888485, AI273419
27	HAVVG36	37	897944	N40203, AA132065, C21544, Z25204, AI222332
32	HELHN47	75	726157	H16917, R99750, R99927, W37841, AA058809, AA262900
36	HLHDL42	46	896650	AA459527, AA493655, AA525222, AA291423, AA292224, AA459296, AA477820, AA477819, AA482607, AA634679, AA434062, AI093119, AI273801, AI276354, AI301537, AI189904, AI654014, AI537880, AI587292
36	HAPQU71	76	864781	AA459527, AA525222, AA459296, AA477819, AA482434, AA482607, AA634679, AA434062, AA781487, AI276354, AI301537
36	HAPQU71	77	752580	AA459527
43	HSYBM41	79	901947	T90427, R25979, R26783, R27189, R27188, R35254, R50925, R70863, R74267, H02216, H06669, H06670, H40092, H40369, H41780, H51292, H51301, H51882, H51894, H61928, N23038, N28700, N33859, N68631, W01548, AA005325, AA027882, AA040832, AA043243, AA043346, AA043573, AA044655, AA148797, AA148528, AA150665, AA223940, AA224086, AA576520, AA665203, AA769018, AA809447, AA810023, AA811441, AA878010, AA886545, AA907519, AA916876, D45317, D45319, N56152, N56519, AA653886, AA218729, AA393565, AA446581, AA446708, AA450355, AA450354, AA453407, AA629981, AA779651, AA779771, AI016461, AI038677, AI095720, D20813, Z38274, Z42207, Z42332, Z42532, Z45060, F01736, F02049, F05255, F13593, AI146571, AI281124, AI358558, AI361131, AI371687, AI494243, AI566474, AI570091, AI192128, AI204438, AI207854
44	HLQGP82	54	898035	R00502, H38743, H85650, H86520, N77865, W00857, AA046738, AA887249, AA663549, T16152
44	HSSDG41	80	425964	T96009, R00502, R48771, R48874, R51102, R52265, R52264, R53399, R53964, R53993, R54847, R81531, R81532, R82184, R82229, H17906, H17907, H38339, H38661, H38743, R93136, R94341, H49045, H49474, H53071, H53175, H62728, H62814, H64497, H69648, H70495, H72255, H77464, H77465, H85650, H86520, H93532, H93772, H93993, H93994, N25894, N30173, N40819, N47783, N54928, N63934, N64034, N67042, N69437, N77865, N98260, W03262, W00857, W69452, W69460, W69536, W69544, AA007372, AA044074, AA044185, AA046738, AA088219, AA088640, AA149582, AA151798, AA158770, AA188377, AA215582, AA215768, AA255719, AA262681
48	HTXKF95	58	891275	AA742405, AA814605, AA831751, AA917582, C01813, AA775165, AI341301, AI418901, AI635420
48	HTXKF95	81	834438	AA742405, AA814605, AA831751, AA917582,

				C01813
51	HAPQT56	82	902207	T53693, T53694, R23643, R35066, W87494, AA533443, AA594172, AA603928, AA614344, AA617718, AA569858, AA740560, AA746624, AA804991, AA804997, AA829811, AA862333, AA864826, AA877343, AA902287, AA878942, AA937062, AA936631, AA961830, AA983420, AA991955, AA991995, AA995511, AI005351, AA642608, AA709070, AA779248, AI032697, AI245599, AI264768, AI266613, AI282722, AI289881, AI291076, AI335628, AI340221, AI369678, AI198965, AI190367, AI268176, AI276207, AI291890, AI312642, AI351218

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Examples

Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample

Each cDNA clone in a cited ATCC deposit is contained in a plasmid vector. Table 1 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The table immediately below correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 1 as being isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

Vector Used to Construct Library	Corresponding Deposited Plasmid
Lambda Zap	pBluescript (pBS)
Uni-Zap XR	pBluescript (pBS)
Zap Express	pBK
lafmid BA	plafmid BA
pSport1	pSport1
pCMVSport 2.0	pCMVSport 2.0
pCMVSport 3.0	pCMVSport 3.0
pCR®2.1	pCR®2.1

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res. 16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., Nucleic Acids Res. 17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., Strategies 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey

Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into E. coli strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS. The S and K refers to the orientation of the polylinker to the T7 and T3 primer sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the f1 origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the f1 ori generates sense strand DNA and in the other, antisense.

Vectors pSport1, pCMVSport 2.0 and pCMVSport 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into E. coli strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993).) Vector lafmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into E. coli strain XL-1 Blue. Vector pCR®2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., Nuc. Acids Res. 16:9677-9686 (1988) and Mead, D. et al., Bio/Technology 9: (1991).) Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 1, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited in Table 1 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone identified in Table 1. Typically, each ATCC deposit sample cited in Table 1 comprises a mixture of approximately equal amounts (by weight) of about 50 plasmid DNAs, each containing a different cDNA clone; but such a deposit sample may include plasmids for more or less than 50 cDNA clones, up to about 500 cDNA clones.

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 1. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to SEQ ID NO:X.

Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with ^{32}P-γ-ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:X (i.e., within the region of SEQ ID NO:X bounded by the 5′ NT and the 3′ NT of the clone defined in Table 1) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 ul of reaction mixture with 0.5 ug of the above cDNA template. A convenient reaction mixture is 1.5-5 mM $MgCl_2$, 0.01% (w/v) gelatin, 20 uM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5′ or 3′ non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5′ and 3′ "RACE" protocols which are well known in the art. For instance, a method similar to 5′ RACE is available for generating the missing 5′ end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993).)

Briefly, a specific RNA oligonucleotide is ligated to the 5′ ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5′ portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5′ phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5′ ends of messenger RNAs. This reaction leaves a 5′ phosphate group at the 5′ end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5′ end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5′ end sequence belongs to the desired gene.

Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:X., according to the method described in Example 1. (See also, Sambrook.)

Example 3: Tissue Distribution of Polypeptide

Tissue distribution of mRNA expression of polynucleotides of the present invention is determined using protocols for Northern blot analysis, described by, among others, Sambrook et al. For example, a cDNA probe produced by the method described in Example 1 is labeled with P^{32} using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for mRNA expression.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70 degree C overnight, and the films developed according to standard procedures.

Example 4: Chromosomal Mapping of the Polynucleotides

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds,95 degree C; 1 minute, 56 degree C; 1 minute, 70 degree C. This cycle is repeated 32 times followed by one 5 minute cycle at 70 degree C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

Example 5: Bacterial Expression of a Polypeptide

A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 ($O.D.^{600}$) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4 degree C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with

high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM immidazole. Immidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4 degree C or frozen at -80 degree C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (lacIq). The origin of replication (oriC) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

Example 6: Purification of a Polypeptide from an Inclusion Body

The following alternative method can be used to purify a polypeptide expressed in *E coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10 degree C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfuidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4 degree C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 um membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive

Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant A_{280} monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5 ug of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

Example 7: Cloning and Expression of a Polypeptide in a Baculovirus Expression System

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral

sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., Virology 170:31-39 (1989).

Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence identified in Table 1, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five ug of a plasmid containing the polynucleotide is co-transfected with 1.0 ug of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One ug of BaculoGold™ virus DNA and 5 ug of the plasmid are mixed in a sterile well of a microtiter plate containing 50 ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27 degrees C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 ul of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 uCi of ^{35}S-methionine and 5 uCi ^{35}S-cysteine (available from Amersham) are added.

The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

Example 8: Expression of a Polypeptide in Mammalian Cells

The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSport 2.0, and pCMVSport 3.0. Mammalian host cells that could be used include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of

interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No.209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the vector does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector

are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five µg of the expression plasmid pC6 a pC4 is cotransfected with 0.5 ug of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of metothrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 uM, 2 uM, 5 uM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 uM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

Example 9: Protein Fusions

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 5; see also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the halflife time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused

protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

Human IgG Fc region:
GGGATCCGGAGCCCAAATCTTCTGACAAAACTCACACATGCCCACCGTGC
CCAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCCAAAA
CCCAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGT
GGTGGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGG
ACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTA
CAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACT
GGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCA
ACCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAG
GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGT
GGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCT
CCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG

GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG
GTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:1)

Example 10: Production of an Antibody from a Polypeptide

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing a polypeptide of the present invention is administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of the secreted protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Köhler et al., Nature 256:495 (1975); Köhler et al., Eur. J. Immunol. 6:511 (1976); Köhler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with polypeptide or, more preferably, with a secreted polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56 degrees C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981).) The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide.

Alternatively, additional antibodies capable of binding to the polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce formation of further protein-specific antibodies.

It will be appreciated that Fab and F(ab')2 and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). Alternatively, secreted protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

Example 11: Production Of Secreted Protein For High-Throughput Screening Assays

The following protocol produces a supernatant containing a polypeptide to be tested. This supernatant can then be used in the Screening Assays described herein.

First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at 2 x 10^5 cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8 or 9, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First, person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degrees C for 6 hours.

While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penstrep, or CHO-5 media (116.6 mg/L of CaCl2 (anhyd); 0.00130

mg/L $CuSO_4$-$5H_2O$; 0.050 mg/L of $Fe(NO_3)_3$-$9H_2O$; 0.417 mg/L of $FeSO_4$-$7H_2O$; 311.80 mg/L of Kcl; 28.64 mg/L of $MgCl_2$; 48.84 mg/L of $MgSO_4$; 6995.50 mg/L of NaCl; 2400.0 mg/L of $NaHCO_3$; 62.50 mg/L of NaH_2PO_4-H_20; 71.02 mg/L of Na_2HPO4; .4320 mg/L of $ZnSO_4$-$7H_2O$; .002 mg/L of Arachidonic Acid ; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitric Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine-H_20; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL-H_20; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL-H_20; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalainine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tryrosine-2Na-$2H_20$; 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; and 0.680 mg/L of Vitamin B_{12}; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; and 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person

B adds 1.5ml appropriate media to each well. Incubate at 37 degrees C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 13-20.

It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide directly (e.g., as a secreted protein) or by the polypeptide inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

Example 12: Construction of GAS Reporter Construct

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, Ann. Rev. Biochem. 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN-a, IFN-g, and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:2)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

Ligand	tyk2	JAKs Jak1	Jak2	Jak3	STATS	GAS(elements) or ISRE
IFN family						
IFN-a/B	+	+	-	-	1,2,3	ISRE
IFN-g		+	+	-	1	GAS (IRF1>Lys6>IFP)
Il-10	+	?	?	-	1,3	
gp130 family						
IL-6 (Pleiotrophic)	+	+	+	?	1,3	GAS (IRF1>Lys6>IFP)
Il-11(Pleiotrophic)	?	+	?	?	1,3	
OnM(Pleiotrophic)	?	+	+	?	1,3	
LIF(Pleiotrophic)	?	+	+	?	1,3	
CNTF(Pleiotrophic)	-/+	+	+	?	1,3	
G-CSF(Pleiotrophic)	?	+	?	?	1,3	
IL-12(Pleiotrophic)	+	-	+	+	1,3	
g-C family						
IL-2 (lymphocytes)	-	+	-	+	1,3,5	GAS
IL-4 (lymph/myeloid)	-	+	-	+	6	GAS (IRF1 = IFP >>Ly6)(IgH)
IL-7 (lymphocytes)	-	+	-	+	5	GAS
IL-9 (lymphocytes)	-	+	-	+	5	GAS
IL-13 (lymphocyte)	-	+	?	?	6	GAS
IL-15	?	+	?	+	5	GAS
gp140 family						
IL-3 (myeloid)	-	-	+	-	5	GAS (IRF1>IFP>>Ly6)
IL-5 (myeloid)	-	-	+	-	5	GAS
GM-CSF (myeloid)	-	-	+	-	5	GAS
Growth hormone family						
GH	?	-	+	-	5	
PRL	?	+/-	+	-	1,3,5	
EPO	?	-	+	-	5	GAS(B-CAS>IRF1=IFP>>Ly6)
Receptor Tyrosine Kinases						
EGF	?	+	+	-	1,3	GAS (IRF1)
PDGF	?	+	+	-	1,3	
CSF-1	?	+	+	-	1,3	GAS (not IRF1)

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 13-14, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

5':GCGCCTCGAGATTTCCCCGAAATCTAGATTTCCCCGAAATGATTTCCCC GAAATGATTTCCCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:3)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

5':<u>CTCGAG</u>ATTTCCCCGAAATCTAGATTTCCCCGAAATGATTTCCCCGAAA TGATTTCCCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCG CCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCT CCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCC TCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCT AGGCTTTTGCAAA<u>AAGCTT</u>:3' (SEQ ID NO:5)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 13-14.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 15 and 16. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, Il-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

Example 13: High-Throughput Screening Assay for T-cell Activity.

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml genticin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required number of cells (10^7 per transfection), and resuspend in OPTI-MEM to a final concentration of 10^7 cells/ml. Then add 1ml of 1×10^7 cells in OPTI-MEM to T25 flask and incubate at 37 degrees C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Genticin, and 1% Pen-Strep. These cells are treated with supernatants containing polypeptides of the invention and/or induced polypeptides of the invention as produced by the protocol described in Example 11.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).

After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at –20 degrees C until SEAP assays are performed according to Example 17. The plates containing the remaining treated cells are placed at 4 degrees C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

The above protocol may be used in the generation of both transient, as well as, stable transfected cells, which would be apparent to those of skill in the art.

Example 14: High-Throughput Screening Assay Identifying Myeloid Activity

The following protocol is used to assess myeloid activity by determining whether polypeptides of the invention proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 12, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest 2x10e^7 U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM $Na_2HPO_4.7H_2O$, 1 mM $MgCl_2$, and 675 uM $CaCl_2$. Incubate at 37 degrees C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degrees C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

These cells are tested by harvesting $1x10^8$ cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of $5x10^5$ cells/ml. Plate 200 ul cells per well in the 96-well plate (or $1x10^5$ cells/well).

Add 50 ul of the supernatant prepared by the protocol described in Example 11. Incubate at 37 degrees C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 17.

Example 15: High-Throughput Screening Assay Identifying Neuronal Activity.

When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat phenochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells can be assessed.

The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO:6)

5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO:7)

Using the GAS:SEAP/Neo vector produced in Example 12, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1 promoter.

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 11. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count

the cell number and add more low serum medium to reach final cell density as $5x10^5$ cells/ml.

Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to $1x10^5$ cells/well). Add 50 ul supernatant produced by Example 11, 37°C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 17.

Example 16: High-Throughput Screening Assay for T-cell Activity

NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF- KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I- KB is phosphorylated and degraded, causing NF- KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- KB include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 11. Activators or inhibitors of NF-KB would be useful in treating diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTTCCC) (SEQ ID NO:8), 18 bp of sequence

complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGAC TTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO:9)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene) Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTCC ATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCC ATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGA CTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTA TTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAA GCTT:3' (SEQ ID NO:10)

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with SalI and NotI.

Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 13. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described

in Example 13. As a positive control, exogenous TNF alpha (0.1,1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

Example 17: Assay for SEAP Activity

As a reporter molecule for the assays described in Examples 13-16, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

Reaction Buffer Formulation:

# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75
14	80	4
15	85	4.25
16	90	4.5
17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6
23	125	6.25
24	130	6.5

25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	170	8.5
33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

Example 18: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability

Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO_2 incubator for

20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4 , 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated at 37 degrees C in a CO_2 incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to 2-5x10^6 cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1x10^6 cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley CellWash with 200 ul, followed by an aspiration step to 100 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-4 . The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event which has resulted in an increase in the intracellular Ca^{++} concentration.

Example 19: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is

unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, the identification of novel human secreted proteins capable of activating tyrosine kinase signal transduction pathways are of interest. Therefore, the following protocol is designed to identify those novel human secreted proteins capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford,MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford,MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in

Example 11, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na3VO4, 2 mM Na4P2O7 and a cocktail of protease inhibitors (# 1836170) obtained from Boeheringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4 degrees C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degrees C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg_{2+} (5mM ATP/50mM $MgCl_2$), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM $MgCl_2$, 5 mM $MnCl_2$, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degrees C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mm EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degrees C for 20

min. This allows the streptavadin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phospotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degrees C for one hour. Wash the well as above.

Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

Example 20: High-Throughput Screening Assay Identifying Phosphorylation Activity

As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 19, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degrees C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and

cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 11 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation.

Example 21: Method of Determining Alterations in a Gene Corresponding to a Polynucleotide

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United

States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenindeoxy-uridine 5'-triphosphate (Boehringer Manheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

Example 22: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in

Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

Example 23: Formulation

The invention also provides methods of treatment and/or prevention diseases, disorders, and/or conditions (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant a polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the Therapeutic is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Therapeutics can be are administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), bucally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), bucally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules), suitable hydrophobic materials

(for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., Biopolymers 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (*see* generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci.(USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation.

For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized

formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diptheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate

administration of one of the compounds or agents given first, followed by the second.

The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines and/or growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokine-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892),TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In

another specific embodiment, Therapeutics of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, Therapeutics of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin,

erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

Conventional nonspecific immunosuppressive agents, that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

In specific embodiments, Therapeutics of the invention are administered in combination with immunosuppressants. Immunosuppressants preparations that may be administered with the Therapeutics of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucorticosteroids, and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the Therapeutics of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

In an additional embodiment, the Therapeutics of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-

acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

In another embodiment, compostions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephalen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

In a specific embodiment, Therapeutics of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, Therapeutics of the invention are administered in combination with Rituximab. In a further embodiment, Therapeutics of the invention are administered with Rituxmab and CHOP, or Rituxmab and any combination of the components of CHOP.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the invention may be administered

with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PlGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PlGF-2), as disclosed in Hauser et al., Gorwth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are incorporated herein by reference herein.

In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but

are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

In additional embodiments, the Therapeutics of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Example 24: Method of Treating Decreased Levels of the Polypeptide

The present invention relates to a method for treating an individual in need of an increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the invention (including polypeptides of the invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a secreted protein in an individual can be treated by administering the polypeptide of the present invention, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a Therapeutic comprising an amount of the polypeptide to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 23.

Example 25: Method of Treating Increased Levels of the Polypeptide

The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of

decreasing levels of a polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer. For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 23.

Example 26: Method of Treatment Using Gene Therapy-Ex Vivo

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is

maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

Example 27: Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides of the Invention

Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA,* 86:8932-8935

(1989); and Zijlstra et al., *Nature,* 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2 HPO_4, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately $3X10^6$ cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3'end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3'end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5'end and a HindIII site at the 3'end. The CMV promoter and the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 μg/ml. 0.5 ml of the cell suspension (containing approximately $1.5.X10^6$ cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 μF and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

Example 28: Method of Treatment Using Gene Therapy - In Vivo

Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide. The polynucleotide of the present invention may be operatively linked to a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., Cardiovasc. Res. 35(3):470-479 (1997); Chao et al., Pharmacol. Res. 35(6):517-522 (1997); Wolff, Neuromuscul. Disord. 7(5):314-318 (1997); Schwartz et al., Gene Ther. 3(5):405-411 (1996); Tsurumi et al., Circulation 94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or

precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg

body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper

dosages and other treatment parameters in humans and other animals using naked DNA.

Example 29: Transgenic Animals.

The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (i.e., polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pleuripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding

strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

Example 30: Knock-Out Animals.

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., Nature 317:230-234 (1985); Thomas & Capecchi, Cell 51:503-512 (1987); Thompson et al., Cell 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (*e.g.*, see Thomas &

Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For

example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

Example 31: Production of an Antibody

a) Hybridoma Technology

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing polypeptide(s) of the invention are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of polypeptide(s) of the invention is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for polypeptide(s) of the invention are prepared using hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with polypeptide(s) of the invention, or, more preferably, with a secreted polypeptide-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 μg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line

(SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide(s) of the invention.

Alternatively, additional antibodies capable of binding polypeptide(s) of the invention can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the polypeptide(s) of the invention protein-specific antibody can be blocked by polypeptide(s) of the invention. Such antibodies comprise anti-idiotypic antibodies to the polypeptide(s) of the invention protein-specific antibody and are used to immunize an animal to induce formation of further polypeptide(s) of the invention protein-specific antibodies.

For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

b) Isolation Of Antibody Fragments Directed polypeptide(s) of the invention From A Library Of scFvs

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against polypeptide(s) of the

invention to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library. A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 109 E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 μg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to innoculate 50 ml of 2xTY-AMP-GLU, 2 x 108 TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 μg/ml ampicillin and 50 ug/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 μg ampicillin/ml and 25 μg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 μm filter (Minisart NML; Sartorius) to give a final concentration of approximately 1013 transducing units/ml (ampicillin-resistant clones).

Panning of the Library. Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 μg/ml or 10 μg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 1013 TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to

stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 µg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders. Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

Example 32: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can,

in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

In Vitro Assay- Purified polypeptides of the invention, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the polypeptides of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed Staphylococcus aureus Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added 10^5 B-cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5 X 10^{-5}M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and 10^{-5} dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with 3H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

In Vivo Assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of a polypeptide of the invention, or truncated forms thereof. Mice

receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with polypeptides of the invention identify the results of the activity of the polypeptides on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with polypeptide is used to indicate whether the polypeptide specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and polypeptide-treated mice.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 33: T Cell Proliferation Assay

Proliferation assay for Resting PBLs.

A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of ^{3}H-thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 microliters per well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 C (1 microgram/ml in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5 x 10^4/well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of TNF Delta and/or TNF Epsilon protein (total volume 200 microliters). Relevant protein buffer and medium alone are controls.

After 48 hr. culture at 37 C, plates are spun for 2 min. at 1000 rpm and 100 microliters of supernatant is removed and stored -20 C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100 microliters of medium containing 0.5 microcuries of ^{3}H-thymidine and cultured at 37 C for 18-24 hr. Wells are harvested and incorporation of ^{3}H-thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of TNF Delta and/or TNF Epsilon proteins.

Alternatively, a proliferation assay on resting PBL (peripheral blood lymphocytes) is measured by the up-take of ^{3}H-thymidine. The assay is performed as follows. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% (Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the plastic, which results in a lower background in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non-adherent cells are collected, washed and used in the proliferation assay. The assay is performed in a 96 well plate using 2 x10^4 cells/well in a final volume of 200 microliters. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector (negative control), IL-2 (*), IFNγ, TNFα, IL-10 and TR2. In addition to the control supernatants, recombinant human IL-2 (R & D Systems, Minneapolois, MN) at a final concentration of 100ng/ml is also used. After 24 hours of culture, each well is pulsed with 1uCi of ^{3}H-thymidine (Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of ^{3}H-thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

(*) The amount of the control cytokines IL-2, IFNγ, TNFα and IL-10 produced in each transfection varies between 300pg to 5ng/ml.

Costimulation assay.

A costimulation assay on resting PBL (peripheral blood lymphocytes) is performed in the presence of immobilized antibodies to CD3 and CD28. The use of antibodies specific for the invariant regions of CD3 mimic the induction of T cell activation that would occur through stimulation of the T cell receptor by an antigen. Cross-linking of the TCR (first signal) in the absence of a costimulatory signal (second signal) causes very low induction of proliferation and will eventually result in a state of "anergy", which is characterized by the absence of growth and inability to produce cytokines. The addition of a costimulatory signal such as an antibody to CD28, which mimics the action of the costimulatory molecule. B7-1 expressed on activated APCs, results in enhancement of T cell responses including cell survival and production of IL-2. Therefore this type of assay allows to detect both positive and negative effects caused by addition of supernatants expressing the proteins of interest on T cell proliferation.

The assay is performed as follows. Ninety-six well plates are coated with 100ng/ml anti-CD3 and 5ug/ml anti-CD28 (Pharmingen, San Diego, CA) in a final volume of 100ul and incubated overnight at 4C. Plates are washed twice with PBS before use. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% FCS(Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the plastic, which results in a lower background in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non adherent cells are collected, washed and used in the proliferation assay. The assay is performed in a 96 well plate using 2×10^4 cells/well in a final volume of 200ul. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector only (negative control), IL-2, IFNγ, TNFα, IL-10 and TR2. In addition to the control supernatants recombinant human IL-2 (R & D Systems, Minneapolis, MN) at a final concentration of 10ng/ml is also used. After 24 hours of culture, each well is

pulsed with 1uCi of ^{3}H-thymidine (Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of ^{3}H-thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

Proliferation assay for preactivated-resting T cells.

A proliferation assay on preactivated-resting T cells is performed on cells that are previously activated with the lectin phytohemagglutinin (PHA). Lectins are polymeric plant proteins that can bind to residues on T cell surface glycoproteins including the TCR and act as polyclonal activators. PBLs treated with PHA and then cultured in the presence of low doses of IL-2 resemble effector T cells. These cells are generally more sensitive to further activation induced by growth factors such as IL-2. This is due to the expression of high affinity IL-2 receptors that allows this population to respond to amounts of IL-2 that are 100 fold lower than what would have an effect on a naïve T cell. Therefore the use of this type of cells might enable to detect the effect of very low doses of an unknown growth factor, that would not be sufficient to induce proliferation on resting (naïve) T cells.

The assay is performed as follows. PBMC are isolated by F/H gradient centrifugation from human peripheral blood, and are cultured in10% FCS(Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD) in the presence of 2ug/ml PHA (Sigma, Saint Louis, MO) for three days. The cells are then washed in PBS and cultured in10% FCS/RPMI in the presence of 5ng/ml of human recombinant IL-2 (R & D Systems, Minneapolis, MN) for 3 days. The cells are washed and rested in starvation medium (1%FCS/RPMI) for16 hours prior to the beginning of the proliferation assay. An aliquot of the cells is analyzed by FACS to determine the percentage of T cells (CD3 positive cells) present; this usually ranges between 93-97% depending on the donor. The assay is performed in a 96 well plate using 2 x10^4 cells/well in a final volume of 200ul. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of in10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector (negative control), IL-2, IFNγ, TNFα, IL-10 and TR2. In addition to the

control supernatants recombinant human IL-2 at a final concentration of 10ng/ml is also used. After 24 hours of culture, each well is pulsed with 1uCi of ^{3}H-thymidine(Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of ^{3}H-thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

The studies described in this example test activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 34: Effect of Polypeptides of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells

Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF-α, causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FCγRII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune

responses. IL-12 strongly influences the development of Thl helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells (10^6/ml) are treated with increasing concentrations of polypeptides of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e..g, R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degreesC. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

<u>Monocyte activation and/or increased survival.</u> Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. Polypeptides, agonists, or antagonists of the invention can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque

gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of 2 x 10^6/ml in PBS containing PI at a final concentration of 5 μg/ml, and then incubaed at room temperature for 5 minutes before FACScan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of $5x10^5$ cells/ml with increasing concentrations of the a polypeptide of the invention and under the same conditions, but in the absence of the polypeptide. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of a polypeptide of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e..g, R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

Oxidative burst. Purified monocytes are plated in 96-w plate at $2\text{-}1x10^5$ cell/well. Increasing concentrations of polypeptides of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is

removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20 µl 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of H_2O_2 produced by the macrophages, a standard curve of a H_2O_2 solution of known molarity is performed for each experiment.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polypeptides, polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 35: Biological Effects of Polypeptides of the Invention

Astrocyte and Neuronal Assays·

Recombinant polypeptides of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate a polypeptide of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA 83*:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of a polypeptide of the invention to

induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

Fibroblast and endothelial cell assays·

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE_2 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or polypeptides of the invention with or without IL-1α for 24 hours. The supernatants are collected and assayed for PGE_2 by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without polypeptides of the invention IL-1α for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or polypeptides of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with polypeptides of the invention.

Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic

projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP^+) and released. Subsequently, MPP^+ is actively accumulated in dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP^+ is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotidamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

Based on the data with FGF-2, polypeptides of the invention can be evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of a polypeptide of the invention is first examined in vitro in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm^2 on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days in vitro and are processed for tyrosine hydroxylase, a specific marker for dopminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons

would represent an increase in the number of dopaminergic neurons surviving *in vitro*. Therefore, if a polypeptide of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the polypeptide may be involved in Parkinson's Disease.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 36: The Effect of Polypeptides of the Invention on the Growth of Vascular Endothelial Cells

On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at $2\text{-}5 \times 10^4$ cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnique, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. A polypeptide having the amino acid sequence of SEQ ID NO:Y, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

An increase in the number of HUVEC cells indicates that the polypeptide of the invention may proliferate vascular endothelial cells.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 37: Stimulatory Effect of Polypeptides of the Invention on the Proliferation of Vascular Endothelial Cells

For evaluation of mitogenic activity of growth factors, the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium) assay with the electron coupling reagent PMS (phenazine methosulfate) was

performed (CellTiter 96 AQ, Promega). Cells are seeded in a 96-well plate (5,000 cells/well) in 0.1 mL serum-supplemented medium and are allowed to attach overnight. After serum-starvation for 12 hours in 0.5% FBS, conditions (bFGF, $VEGF_{165}$ or a polypeptide of the invention in 0.5% FBS) with or without Heparin (8 U/ml) are added to wells for 48 hours. 20 mg of MTS/PMS mixture (1:0.05) are added per well and allowed to incubate for 1 hour at 37°C before measuring the absorbance at 490 nm in an ELISA plate reader. Background absorbance from control wells (some media, no cells) is subtracted, and seven wells are performed in parallel for each condition. See, Leak *et al.* *In Vitro Cell. Dev. Biol. 30A:*512-518 (1994).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 38: Inhibition of PDGF-induced Vascular Smooth Muscle Cell Proliferation Stimulatory Effect

HAoSMC proliferation can be measured, for example, by BrdUrd incorporation. Briefly, subconfluent, quiescent cells grown on the 4-chamber slides are transfected with CRP or FITC-labeled AT2-3LP. Then, the cells are pulsed with 10% calf serum and 6 mg/ml BrdUrd. After 24 h, immunocytochemistry is performed by using BrdUrd Staining Kit (Zymed Laboratories). In brief, the cells are incubated with the biotinylated mouse anti-BrdUrd antibody at 4 degrees C for 2 h after being exposed to denaturing solution and then incubated with the streptavidin-peroxidase and diaminobenzidine. After counterstaining with hematoxylin, the cells are mounted for microscopic examination, and the BrdUrd-positive cells are counted. The BrdUrd index is calculated as a percent of the BrdUrd-positive cells to the total cell number. In addition, the simultaneous detection of the BrdUrd staining (nucleus) and the FITC uptake (cytoplasm) is performed for individual cells by the concomitant use of bright field illumination and dark field-UV fluorescent illumination. See, Hayashida et al., J. Biol. Chem. 6:271(36):21985-21992 (1996).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 39: Stimulation of Endothelial Migration

This example will be used to explore the possibility that a polypeptide of the invention may stimulate lymphatic endothelial cell migration.

Endothelial cell migration assays are performed using a 48 well microchemotaxis chamber (Neuroprobe Inc., Cabin John, MD; Falk, W., et al., J. Immunological Methods 1980;33:239-247). Polyvinylpyrrolidone-free polycarbonate filters with a pore size of 8 um (Nucleopore Corp. Cambridge, MA) are coated with 0.1% gelatin for at least 6 hours at room temperature and dried under sterile air. Test substances are diluted to appropriate concentrations in M199 supplemented with 0.25% bovine serum albumin (BSA), and 25 ul of the final dilution is placed in the lower chamber of the modified Boyden apparatus. Subconfluent, early passage (2-6) HUVEC or BMEC cultures are washed and trypsinized for the minimum time required to achieve cell detachment. After placing the filter between lower and upper chamber, 2.5×10^5 cells suspended in 50 ul M199 containing 1% FBS are seeded in the upper compartment. The apparatus is then incubated for 5 hours at 37°C in a humidified chamber with 5% CO2 to allow cell migration. After the incubation period, the filter is removed and the upper side of the filter with the non-migrated cells is scraped with a rubber policeman. The filters are fixed with methanol and stained with a Giemsa solution (Diff-Quick, Baxter, McGraw Park, IL). Migration is quantified by counting cells of three random high-power fields (40x) in each well, and all groups are performed in quadruplicate.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 40: Stimulation of Nitric Oxide Production by Endothelial Cells

Nitric oxide released by the vascular endothelium is believed to be a mediator of vascular endothelium relaxation. Thus, activity of a polypeptide of the invention can be assayed by determining nitric oxide production by endothelial cells in response to the polypeptide.

Nitric oxide is measured in 96-well plates of confluent microvascular endothelial cells after 24 hours starvation and a subsequent 4 hr exposure to various levels of a positive control (such as VEGF-1) and the polypeptide of the invention. Nitric oxide in the medium is determined by use of the Griess reagent to measure total nitrite after reduction of nitric oxide-derived nitrate by nitrate reductase. The effect of the polypeptide of the invention on nitric oxide release is examined on HUVEC.

Briefly, NO release from cultured HUVEC monolayer is measured with a NO-specific polarographic electrode connected to a NO meter (Iso-NO, World Precision Instruments Inc.) (1049). Calibration of the NO elements is performed according to the following equation:

$$2\ KNO_2 + 2\ KI + 2\ H_2SO_4\ 6\ 2\ NO + I_2 + 2\ H_2O + 2\ K_2SO_4$$

The standard calibration curve is obtained by adding graded concentrations of KNO_2 (0, 5, 10, 25, 50, 100, 250, and 500 nmol/L) into the calibration solution containing KI and H_2SO_4. The specificity of the Iso-NO electrode to NO is previously determined by measurement of NO from authentic NO gas (1050). The culture medium is removed and HUVECs are washed twice with Dulbecco's phosphate buffered saline. The cells are then bathed in 5 ml of filtered Krebs-Henseleit solution in 6-well plates, and the cell plates are kept on a slide warmer (Lab Line Instruments Inc.) To maintain the temperature at 37°C. The NO sensor probe is inserted vertically into the wells, keeping the tip of the electrode 2 mm under the surface of the solution, before addition of the different conditions. S-nitroso acetyl penicillamin (SNAP) is used as a positive control. The amount of released NO is expressed as picomoles per $1x10^6$ endothelial cells. All values reported are means of four to six measurements in each group (number of cell culture wells). See, Leak *et al. Biochem. and Biophys. Res. Comm. 217*:96-105 (1995).

The studies described in this example tested activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to

test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 41: Effect of Polypepides of the Invention on Cord Formation in Angiogenesis

Another step in angiogenesis is cord formation, marked by differentiation of endothelial cells. This bioassay measures the ability of microvascular endothelial cells to form capillary-like structures (hollow structures) when cultured *in vitro*.

CADMEC (microvascular endothelial cells) are purchased from Cell Applications, Inc. as proliferating (passage 2) cells and are cultured in Cell Applications' CADMEC Growth Medium and used at passage 5. For the *in vitro* angiogenesis assay, the wells of a 48-well cell culture plate are coated with Cell Applications' Attachment Factor Medium (200 ml/well) for 30 min. at 37°C. CADMEC are seeded onto the coated wells at 7,500 cells/well and cultured overnight in Growth Medium. The Growth Medium is then replaced with 300 mg Cell Applications' Chord Formation Medium containing control buffer or a polypeptide of the invention (0.1 to 100 ng/ml) and the cells are cultured for an additional 48 hr. The numbers and lengths of the capillary-like chords are quantitated through use of the Boeckeler VIA-170 video image analyzer. All assays are done in triplicate.

Commercial (R&D) VEGF (50 ng/ml) is used as a positive control. b-esteradiol (1 ng/ml) is used as a negative control. The appropriate buffer (without protein) is also utilized as a control.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 42: Angiogenic Effect on Chick Chorioallantoic Membrane

Chick chorioallantoic membrane (CAM) is a well-established system to examine angiogenesis. Blood vessel formation on CAM is easily visible and quantifiable. The

ability of polypeptides of the invention to stimulate angiogenesis in CAM can be examined.

Fertilized eggs of the White Leghorn chick (*Gallus gallus*) and the Japanese qual (*Coturnix coturnix*) are incubated at 37.8°C and 80% humidity. Differentiated CAM of 16-day-old chick and 13-day-old qual embryos is studied with the following methods.

On Day 4 of development, a window is made into the egg shell of chick eggs. The embryos are checked for normal development and the eggs sealed with cellotape. They are further incubated until Day 13. Thermanox coverslips (Nunc, Naperville, IL) are cut into disks of about 5 mm in diameter. Sterile and salt-free growth factors are dissolved in distilled water and about 3.3 mg/ 5 ml are pipetted on the disks. After air-drying, the inverted disks are applied on CAM. After 3 days, the specimens are fixed in 3% glutaraldehyde and 2% formaldehyde and rinsed in 0.12 M sodium cacodylate buffer. They are photographed with a stereo microscope [Wild M8] and embedded for semi- and ultrathin sectioning as described above. Controls are performed with carrier disks alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 43: Angiogenesis Assay Using a Matrigel Implant in Mouse

In vivo angiogenesis assay of a polypeptide of the invention measures the ability of an existing capillary network to form new vessels in an implanted capsule of murine extracellular matrix material (Matrigel). The protein is mixed with the liquid Matrigel at 4 degree C and the mixture is then injected subcutaneously in mice where it solidifies. After 7 days, the solid "plug" of Matrigel is removed and examined for the presence of new blood vessels. Matrigel is purchased from Becton Dickinson Labware/Collaborative Biomedical Products.

When thawed at 4 degree C the Matrigel material is a liquid. The Matrigel is mixed with a polypeptide of the invention at 150 ng/ml at 4 degrees C and drawn into cold 3 ml syringes. Female C57Bl/6 mice approximately 8 weeks old are injected with the

mixture of Matrigel and experimental protein at 2 sites at the midventral aspect of the abdomen (0.5 ml/site). After 7 days, the mice are sacrificed by cervical dislocation, the Matrigel plugs are removed and cleaned (i.e., all clinging membranes and fibrous tissue is removed). Replicate whole plugs are fixed in neutral buffered 10% formaldehyde, embedded in paraffin and used to produce sections for histological examination after staining with Masson's Trichrome. Cross sections from 3 different regions of each plug are processed. Selected sections are stained for the presence of vWF. The positive control for this assay is bovine basic FGF (150 ng/ml). Matrigel alone is used to determine basal levels of angiogenesis.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 44: Rescue of Ischemia in Rabbit Lower Limb Model

To study the in vivo effects of polynucleotides and polypeptides of the invention on ischemia, a rabbit hindlimb ischemia model is created by surgical removal of one femoral arteries as described previously (Takeshita *et al.*, *Am J. Pathol 147*:1649-1660 (1995)). The excision of the femoral artery results in retrograde propagation of thrombus and occlusion of the external iliac artery. Consequently, blood flow to the ischemic limb is dependent upon collateral vessels originating from the internal iliac artery (Takeshita*et al.* *Am J. Pathol 147*:1649-1660 (1995)). An interval of 10 days is allowed for post-operative recovery of rabbits and development of endogenous collateral vessels. At 10 day post-operatively (day 0), after performing a baseline angiogram, the internal iliac artery of the ischemic limb is transfected with 500 mg naked expression plasmid containing a polynucleotide of the invention by arterial gene transfer technology using a hydrogel-coated balloon catheter as described (Riessen *et al. Hum Gene Ther. 4*:749-758 (1993); Leclerc *et al. J. Clin. Invest. 90*: 936-944 (1992)). When a polypeptide of the invention is used in the treatment, a single bolus of 500 mg polypeptide of the invention or control is delivered into the internal iliac artery of the ischemic limb over a period of 1

min. through an infusion catheter. On day 30, various parameters are measured in these rabbits: (a) BP ratio - The blood pressure ratio of systolic pressure of the ischemic limb to that of normal limb; (b) Blood Flow and Flow Reserve - Resting FL: the blood flow during undilated condition and Max FL: the blood flow during fully dilated condition (also an indirect measure of the blood vessel amount) and Flow Reserve is reflected by the ratio of max FL: resting FL; (c) Angiographic Score - This is measured by the angiogram of collateral vessels. A score is determined by the percentage of circles in an overlaying grid that with crossing opacified arteries divided by the total number m the rabbit thigh; (d) Capillary density - The number of collateral capillaries determined in light microscopic sections taken from hindlimbs.

The studies described in this example tested activity of polynucleotides and polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the agonists, and/or antagonists of the invention.

Example 45: Effect of Polypeptides of the Invention on Vasodilation

Since dilation of vascular endothelium is important in reducing blood pressure, the ability of polypeptides of the invention to affect the blood pressure in spontaneously hypertensive rats (SHR) is examined. Increasing doses (0, 10, 30, 100, 300, and 900 mg/kg) of the polypeptides of the invention are administered to 13-14 week old spontaneously hypertensive rats (SHR). Data are expressed as the mean +/- SEM. Statistical analysis are performed with a paired t-test and statistical significance is defined as $p<0.05$ vs. the response to buffer alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 46: Rat Ischemic Skin Flap Model

The evaluation parameters include skin blood flow, skin temperature, and factor VIII immunohistochemistry or endothelial alkaline phosphatase reaction. Expression of

polypeptides of the invention, during the skin ischemia, is studied using in situ hybridization.

The study in this model is divided into three parts as follows:

a) Ischemic skin

b) Ischemic skin wounds

c) Normal wounds

The experimental protocol includes:

a) Raising a 3x4 cm, single pedicle full-thickness random skin flap (myocutaneous flap over the lower back of the animal).

b) An excisional wounding (4-6 mm in diameter) in the ischemic skin (skin-flap).

c) Topical treatment with a polypeptide of the invention of the excisional wounds (day 0, 1, 2, 3, 4 post-wounding) at the following various dosage ranges: 1mg to 100 mg.

d) Harvesting the wound tissues at day 3, 5, 7, 10, 14 and 21 post-wounding for histological, immunohistochemical, and in situ studies.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 47: Peripheral Arterial Disease Model

Angiogenic therapy using a polypeptide of the invention is a novel therapeutic strategy to obtain restoration of blood flow around the ischemia in case of peripheral arterial diseases. The experimental protocol includes:

a) One side of the femoral artery is ligated to create ischemic muscle of the hindlimb, the other side of hindlimb serves as a control.

b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-3 weeks.

c) The ischemic muscle tissue is collected after ligation of the femoral

artery at 1, 2, and 3 weeks for the analysis of expression of a polypeptide of the invention and histology. Biopsy is also performed on the other side of normal muscle of the contralateral hindlimb.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 48: Ischemic Myocardial Disease Model

A polypeptide of the invention is evaluated as a potent mitogen capable of stimulating the development of collateral vessels, and restructuring new vessels after coronary artery occlusion. Alteration of expression of the polypeptide is investigated in situ. The experimental protocol includes:

a) The heart is exposed through a left-side thoracotomy in the rat. Immediately, the left coronary artery is occluded with a thin suture (6-0) and the thorax is closed.

b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-4 weeks.

c) Thirty days after the surgery, the heart is removed and cross-sectioned for morphometric and in situ analyzes.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 49: Rat Corneal Wound Healing Model

This animal model shows the effect of a polypeptide of the invention on neovascularization. The experimental protocol includes:

a) Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.

b) Inserting a spatula below the lip of the incision facing the outer corner of the eye.

c) Making a pocket (its base is 1-1.5 mm form the edge of the eye).

d) Positioning a pellet, containing 50ng- 5ug of a polypeptide of the invention, within the pocket.

e) Treatment with a polypeptide of the invention can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 50: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models

A. Diabetic db+/db+ Mouse Model.

To demonstrate that a polypeptide of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, M.H. *et al., J. Surg. Res. 52*:389 (1992); Greenhalgh, D.G. *et al., Am. J. Pathol. 136*:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al. Proc. Natl. Acad. Sci. USA 77*:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al., J. Immunol. 120*:1375 (1978); Debray-Sachs, M. *et al., Clin. Exp. Immunol. 51(1)*:1-7 (1983); Leiter *et al., Am. J. of Pathol. 114*:46-55 (1985)). Peripheral neuropathy, myocardial complications, and

microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. *et al., Exp. Neurol. 83(2)*:221-232 (1984); Robertson *et al., Diabetes 29(1)*:60-67 (1980); Giacomelli *et al., Lab Invest. 40(4)*:460-473 (1979); Coleman, D.L., *Diabetes 31 (Suppl)*:1-6 (1982)). These homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel *et al., J. Immunol. 120*:1375-1377 (1978)).

The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, *et al., Am. J. of Pathol. 136*:1235-1246 (1990)).

Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study. Animals are individually housed and received food and water ad libitum. All manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med. 172*:245-251 (1990)). Briefly, on the day of wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically

using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

A polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is $64mm^2$, the corresponding size of the dermal punch. Calculations are made using the following formula:

[Open area on day 8] - [Open area on day 1] / [Open area on day 1]

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with a polypeptide of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al., Am. J. Pathol. 136:*1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte

growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer can serve as a positive tissue control and human brain tissue can be used as a negative tissue control. Each specimen includes a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

B. Steroid Impaired Rat Model

The inhibition of wound healing by steroids has been well documented in various *in vitro* and *in vivo* systems (Wahl, Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahl*et al., J. Immunol. 115*: 476-481 (1975); Werb *et al., J. Exp. Med. 147*:1684-1694 (1978)). Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert *et al., An. Intern. Med. 37*:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck *et al., Growth Factors. 5:* 295-304 (1991); Haynes *et al., J. Clin. Invest. 61:* 703-797 (1978)) and producing a transient reduction of circulating monocytes (Haynes et al., *J. Clin. Invest. 61:* 703-797 (1978); Wahl, "Glucocorticoids and wound healing", *In:* Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck *et al., Growth Factors. 5:* 295-304 (1991); Haynes *et al., J. Clin. Invest. 61:* 703-797 (1978); Wahl, "Glucocorticoids and wound healing", *In*: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989); Pierce *et al., Proc. Natl. Acad. Sci. USA 86*: 2229-2233 (1989)).

To demonstrate that a polypeptide of the invention can accelerate the healing process, the effects of multiple topical applications of the polypeptide on full thickness

excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad libitum*. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

The wounding protocol is followed according to section A, above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

The polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is $64mm^2$, the corresponding size of the dermal punch. Calculations are made using the following formula:

[Open area on day 8] - [Open area on day 1] / [Open area on day 1]

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin is improved by treatment with a polypeptide of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 51: Lymphadema Animal Model

or The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of a polypeptide of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and

histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosis and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing

perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

Circumference Measurements: Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

Volumetric Measurements: On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and Ca2+ comparison.

Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

<u>Example 52: Suppression of TNF alpha-induced adhesion molecule expression by a Polypeptide of the Invention</u>

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules

(CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Tumor necrosis factor alpha (TNF-a), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

The potential of a polypeptide of the invention to mediate a suppression of TNF-a induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF-a treated ECs when co-stimulated with a member of the FGF family of proteins.

To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO_2. HUVECs are seeded in 96-well plates at concentrations of 1 x 10^4 cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90 ul of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 ul volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are

aspirated to remove medium and 100 µl of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10 µl of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 µg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

Then add 20 µl of diluted ExtrAvidin-Alkaline Phosphotase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer: 1:5,000 (10^0) > $10^{-0.5}$ > 10^{-1} > $10^{-1.5}$. 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNNP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 53: Assay for the Stimulation of Bone Marrow CD34+ Cell Proliferation

This assay is based on the ability of human CD34+ to proliferate in the presence of hematopoietic growth factors and evaluates the ability of isolated polypeptides expressed in mammalian cells to stimulate proliferation of CD34+ cells.

It has been previously shown that most mature precursors will respond to only a single signal. More immature precursors require at least two signals to respond. Therefore, to test the effect of polypeptides on hematopoietic activity of a wide range of progenitor cells, the assay contains a given polypeptide in the presence or absence of other hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor (SCF) in combination with tested sample. SCF alone has a very limited effect on the proliferation of bone marrow (BM) cells, acting in such conditions only as a "survival" factor. However, combined with any factor exhibiting stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested polypeptide has a stimulatory effect on a hematopoietic progenitors, such activity can be easily detected. Since normal BM cells have a low level of cycling cells, it is likely that any inhibitory effect of a given polypeptide, or agonists or antagonists thereof, might not be detected. Accordingly, assays for an inhibitory effect on progenitors is preferably tested in cells that are first subjected to *in vitro* stimulation with SCF+IL+3, and then contacted with the compound that is being evaluated for inhibition of such induced proliferation.

Briefly, CD34+ cells are isolated using methods known in the art. The cells are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-glutamine (500ml) Quality Biological, Inc., Gaithersburg, MD Cat# 160-204-101). After several gentle centrifugation steps at 200 x g, cells are allowed to rest for one hour. The cell count is adjusted to 2.5 x 10^5 cells/ml. During this time, 100 μl of sterile water is added to the peripheral wells of a 96-well plate. The cytokines that can be tested with a given polypeptide in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and in combination with rhSCF and rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one hour, 10 μl of prepared cytokines, 50 μl SID (supernatants at 1:2 dilution = 50 μl) and 20 μl of diluted cells are added to the media which is already present in the wells to allow for a final total volume of 100 μl. The plates are then placed in a 37°C/5% CO_2 incubator for five days.

Eighteen hours before the assay is harvested, 0.5 μCi/well of [3H] Thymidine is added in a 10 μl volume to each well to determine the proliferation rate. The

experiment is terminated by harvesting the cells from each 96-well plate to a filtermat using the Tomtec Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60 µl Microscint is added to each well and the plate sealed with TopSeal-A press-on sealing film A bar code 15 sticker is affixed to the first plate for counting. The sealed plates is then loaded and the level of radioactivity determined via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

The studies described in this example test the activity of a given polypeptide to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof. As a nonlimiting example, potential antagonists tested in this assay would be expected to inhibit cell proliferation in the presence of cytokines and/or to increase the inhibition of cell proliferation in the presence of cytokines and a given polypeptide. In contrast, potential agonists tested in this assay would be expected to enhance cell proliferation and/or to decrease the inhibition of cell proliferation in the presence of cytokines and a given polypeptide.

The ability of a gene to stimulate the proliferation of bone marrow CD34+ cells indicates that polynucleotides and polypeptides corresponding to the gene are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein.

__Example 54: Assay for Extracellular Matrix Enhanced Cell Response (EMECR)__

The objective of the Extracellular Matrix Enhanced Cell Response (EMECR) assay is to identify gene products (e.g., isolated polypeptides) that act on the hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM.

Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in *in vitro* suspension culture. The ability of stem cells to undergo self-renewal *in vitro* is dependent upon their interaction with the stromal cells and the ECM protein fibronectin (fn). Adhesion of cells to fn is mediated by the $\alpha_5.\beta_1$ and $\alpha_4.\beta_1$ integrin receptors, which are expressed by human and mouse hematopoietic stem cells. The factor(s) which integrate with the ECM environment and responsible for stimulating stem cell self-renewal has not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with fn fragment at a coating concentration of 0.2 μg/ cm^2. Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control, conditions under which little self-renewal but pronounced differentiation of the stem cells is to be expected. Gene products are tested with appropriate negative controls in the presence and absence of SCF(5.0 ng/ml), where test factor supernates represent 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment (5% CO_2, 7% O_2, and 88% N_2) tissue culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA. Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACScan.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

If a particular gene product is found to be a stimulator of hematopoietic progenitors, polynucleotides and polypeptides corresponding to the gene may be useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the “Immune Activity” and “Infectious Disease” sections above, and elsewhere herein. The gene product may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Additionally, the polynucleotides and/or polypeptides of the gene of interest and/or agonists and/or antagonists thereof, may also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

Moreover, polynucleotides and polypeptides corresponding to the gene of interest may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, for example, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

Example 55: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation

The polypeptide of interest is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-assays are performed with each sample. The first assay examines the effect of the polypeptide of interest on the proliferation of normal human dermal fibroblasts (NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without co-TNFa stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100 μl culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin,

2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5 μg/ml hEGF, 5mg/ml insulin, 1μg/ml hFGF, 50mg/ml gentamycin, 50 μg/ml Amphotericin B, 5%FBS. After incubation @ 37°C for at least 4-5 hours culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains fibroblast basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for AoSMC contains SM basal media, 50mg/ml gentamycin, 50μg/ml Amphotericin B, 0.4% FBS. Incubate at 37C until day 2.

On day 2, serial dilutions and templates of the polypeptide of interest are designed which should always include media controls and known-protein controls. For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNFa is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml (AoSMC). Then add 1/3 vol media containing controls or supernatants and incubate at 37C/5% CO_2 until day 5.

Transfer 60μl from each well to another labeled 96-well plate, cover with a plate-sealer, and store at 4C until Day 6 (for IL6 ELISA). To the remaining 100 μl in the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the culture volume (10μl). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100 ul/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200 μl/well of Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50 μl/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker.

Wash plates with wash buffer and blot on paper towels. Dilute EU-labeled Streptavidin 1:1000 in Assay buffer, and add 100 µl/well. Cover the plate and incubate 1 h at RT. Wash plates with wash buffer. Blot on paper towels.

Add 100 µl/well of Enhancement Solution. Shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay were tabulated and averaged.

A positive result in this assay suggests AoSMC cell proliferation and that the gene product of interest may be involved in dermal fibroblast proliferation and/or smooth muscle cell proliferation. A positive result also suggests many potential uses of polypeptides, polynucleotides, agonists and/or antagonists of the gene/gene product of interest. For example, inflammation and immune responses, wound healing, and angiogenesis, as detailed throughout this specification. Particularly, polypeptides of the gene product and polynucleotides of the gene may be used in wound healing and dermal regeneration, as well as the promotion of vasculargenesis, both of the blood vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, antagonists of polypeptides of the gene product and polynucleotides of the gene may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; artheroscleric plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uvietis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, antagonists of polypeptides of the

gene product and polynucleotides of the gene may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

Example 56: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100 µl of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 µl volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 µl of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10 µl of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 µg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are

incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20 μl of diluted ExtrAvidin-Alkaline Phosphotase (1:5,000 dilution, refered to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100 μl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer: 1:5,000 (10^0) > $10^{-0.5}$ > 10^{-1} > $10^{-1.5}$. 5 μl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 μl of pNNP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50 μl of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

Example 57: Alamar Blue Endothelial Cells Proliferation Assay

This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration. Dilutions of the protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37-C overnight. After the overnight incubation of the cells, the growth media is removed and replaced with

GIBCO EC-SFM. The cells are treated with the appropriate dilutions of the protein of interest or control protein sample(s) (prepared in SFM) in triplicate wells with additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days. After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

Alamar blue is an oxidation-reduction indicator that both fluoresces and changes color in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form. i.e. stimulated proliferation will produce a stronger signal and inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity. The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

Example 58: Detection of Inhibition of a Mixed Lymphocyte Reaction

This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by gene products (e.g., isolated polypeptides). Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by these polypeptides since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

Polypeptides of interest found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes,

inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM®, density 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to 2 x 10^6 cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to 2 x 10^5 cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of test materials (50 μl) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1 μg/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10 μg/ml. Cells are cultured for 7-8 days at 37°C in 5% CO_2, and 1 μC of [^{3}H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other

disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their entireties. Additionally, the contents of U.S. applications Serial Nos. 60/152,317 and 60/152,315 are all hereby incorporated by reference in their entirety.

Applicant's or agent's file reference number	PZ043PCT	International application No.	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 144, line N/A.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
02 September 1999	PTA-623

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).

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CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

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ATCC Deposit No.: PTA-623
Page No. 3

DENMARK

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SWEDEN

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NETHERLANDS

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Applicant's or agent's file reference number	PZ043PCT	International application No.	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 144, line N/A.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
02 September 1999	PTA-622

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).

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Page No. 2

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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FINLAND

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UNITED KINGDOM

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ATCC Deposit No.: PTA-622
Page No. 3

DENMARK

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NETHERLANDS

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Applicant's or agent's file reference number	PZ043PCT	International application No.	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 145, line N/A.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
20 September 1999	PTA-725

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
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CANADA

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FINLAND

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UNITED KINGDOM

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ATCC Deposit No.: PTA-725
Page No. 3

DENMARK

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NETHERLANDS

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Applicant's or agent's file reference number	PZ043PCT	International application No.	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 145, line N/A.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
07 May 1998	209852

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
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CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

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FINLAND

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UNITED KINGDOM

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ATCC Deposit No.: 209852
Page No. 3

DENMARK

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NETHERLANDS

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Applicant's or agent's file reference number	PZ043PCT	International application No.	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 145, line N/A.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
14 November 1997	209463

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).
Continued on the Attached Pages 2 & 3

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☑ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer Virginia Lily	Authorized officer

ATCC Deposit No. 209463
Page No. 2

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: 209463
Page No. 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PZ043PCT	International application No.	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 146, line N/A.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
22 May 1997	209076

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).
Continued on the Attached Pages 2 & 3

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☑ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer Virginia [signature]	Authorized officer

Form PCT/RO/134 (July 1992)

ATCC Deposit No. 209076
Page No. 2

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: 209076
Page No. 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PZ043PCT	International application No.	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 146, line N/A.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
21 September 1999	PTA-736

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).
Continued on the Attached Pages 2 & 3

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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Form PCT/RO/134 (July 1992)

ATCC Deposit No. PTA-736
Page No. 2

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: PTA-736
Page No. 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PZ043PCT	International application No.	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 148, line N/A.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
07 June 1999	PTA-181

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).

Continued on the Attached Pages 2 & 3

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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Authorized officer Virginia [signature]	Authorized officer

ATCC Deposit No. PTA-181
Page No. 2

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: PTA-181
Page No. 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PZ043PCT	International application No.	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 148, line N/A.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
08 April 1999	203917

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).

Continued on the Attached Pages 2 & 3

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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Authorized officer Virginia Ely	Authorized officer

Form PCT/RO/134 (July 1992)

ATCC Deposit No. 203917
Page No. 2

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: 203917
Page No. 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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NETHERLANDS

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Applicant's or agent's file reference number	PZ043PCT	International application No.	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 149, line N/A.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
11 August 1999	PTA-499

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).

Continued on the Attached Pages 2 & 3

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Authorized officer Virginia Lily	Authorized officer

ATCC Deposit No. PTA-499
Page No. 2

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: PTA-499
Page No. 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PZ043PCT	International application No.	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 149, line N/A.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
24 November 1999	PTA-987

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).
Continued on the Attached Pages 2 & 3

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☑ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer Virginia Lily	Authorized officer

Form PCT/RO/134 (July 1992)

ATCC Deposit No. PTA-987
Page No. 2

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: PTA-987
Page No. 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PZ043PCT	International application No.	UNASSIGNED

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 150, line N/A.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
02 November 1999	PTA-909

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).
Continued on the Attached Pages 2 & 3

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☑ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer Virginia Ely	Authorized officer

ATCC Deposit No. PTA-909
Page No. 2

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

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UNITED KINGDOM

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Page No. 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO:X or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;

(b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:Y or a polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;

(c) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y or a polypeptide domain encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;

(d) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or a polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;

(e) a polynucleotide encoding a polypeptide of SEQ ID NO:Y or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X, having biological activity;

(f) a polynucleotide which is a variant of SEQ ID NO:X;

(g) a polynucleotide which is an allelic variant of SEQ ID NO:X;

(h) a polynucleotide which encodes a species homologue of the SEQ ID NO:Y;

(i) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.

2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a secreted protein.

3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.

4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:X or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.

5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.

9. A recombinant host cell produced by the method of claim 8.

10. The recombinant host cell of claim 9 comprising vector sequences.

11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(b) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z, having biological activity;

(c) a polypeptide domain of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(d) a polypeptide epitope of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(e) a secreted form of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(f) a full length protein of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(g) a variant of SEQ ID NO:Y;

(h) an allelic variant of SEQ ID NO:Y; or

(i) a species homologue of the SEQ ID NO:Y.

12. The isolated polypeptide of claim 11, wherein the secreted form or the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

14. A recombinant host cell that expresses the isolated polypeptide of claim 11.

15. A method of making an isolated polypeptide comprising:

(a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and

(b) recovering said polypeptide.

16. The polypeptide produced by claim 15.

17. A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

19. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

20. A method for identifying a binding partner to the polypeptide of claim 11 comprising:

(a) contacting the polypeptide of claim 11 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

21. The gene corresponding to the cDNA sequence of SEQ ID NO:Y.

22. A method of identifying an activity in a biological assay, wherein the method comprises:

(a) expressing SEQ ID NO:X in a cell;

(b) isolating the supernatant;

(c) detecting an activity in a biological assay; and

(d) identifying the protein in the supernatant having the activity.

23. The product produced by the method of claim 20.

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<211> 1038
<212> DNA
<213> Homo sapiens
```

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<220>
<221> SITE
<222> (963)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1025)
<223> n equals a,t,g, or c

<400> 17
gcaacaagag ccacctctgg gtgraggagg aggtctggcg gatggagatc tacctctccc      60
tgggagtgct ggccctcggc acgttgtccc tgctggccgt gacctcactg ccgtccattg     120
caaactcgct caactggagg gagttcagct tcgttcagtc ctcactgggc tttgtggccc     180
tcgtgctgag cacactgcac acgctcacct acggctggac ccgcgccttc gaggagagcc     240
gctacaagtt ctacctgcct cccaccttca cgctcacgct gctggtgccc tgcgtcgtca     300
tcctggccaa agccctgttt ctcctgccct gcatcagccg cagactcgcc aggatccgga     360
gaggctggga gagggagagc accatcaagt tcacgctgcc cacagaccac gccctggccg     420
agaagacgag ccacgtatga ggtgcctgcc ctgggctctg gaccccgggc acacgaggga     480
cggtgccctg agcccgttag gttttctttt cttggtggtg caaagtggta taactgtgtg     540
caaataggag gtttgaggtc caaattcctg ggactcaaat gtatgcagta ctattcagaa     600
tgatatacac acatatgtgt atatgtattt acatatattc cacatatata acaggatttg     660
caattataca tagctagcta aaaagttggg tctctgagat ttcaacttgt agatttaaaa     720
acaagtgccg tacgttaaga gaagagcaga tcatgctatt gtgacatttg cagagatata     780
cacacacttt ttgtacagaa gaggcttgtg ctgtggtggg ttcgatttat ccctgcccac     840
cccaycccca caacttccct tttgctactt ccccaaggct cttgcagagc tagggctctg     900
aaggggaggg aaggcaacgg ctctgcccag agccatcctg gagcatgtga gcagcgctgg     960
ctntttcctc caccttgggc agcacaggag gcctgggagg gggaaatcag cagtcggccc    1020
tgagntttgc ctggcccc                                                  1038

<210> 18
<211> 718
<212> DNA
<213> Homo sapiens

<400> 18
ggcacgagct cagccacgtg accaaccggg tcacatggcc cgcgggacaa catggctgcg      60
cccgcactag ggctggtgtg tggacgttgc cctgagctgg gtctcgtcct cttgctgctg     120
ctgctctcgc tgctgtgtgg agcggcaggg agccaggagg ccgggaccgg tgcgggcgcg     180
gggtcccttg cgggttcttg cggctgcggc acgccccagc ggcctggcgc ccatggcagt     240
tcggcagccg ctcaccgata ctcgcgggag gctaacgctc cgggccccgt acccggagag     300
cggcaactcg cgcactcaaa ggtgctccat cgattcctcc gargcgggtk ggggctgctc     360
ggttcctgga cggggttgga gtaggcaaag caaggcacta gtaggaaggg aagtaaaggt     420
tataaccaca ccccaaatcg agcacctgct gttcccgtat gaggagttcc ttctccgtgc     480
ctcaccggaa gactccattt mattgaccat tagggagttt ggtttgaggg ttattgttac     540
ttctttaccc cctatttctt tctccctcca acctgttctc ttaatgagga tctcataatt     600
ttaaggcaat caaattatgg tttaaatcac catttcctct cttattaacg gaataaatta     660
ggatcctggg tctcagtatc ttcaccaggg ttgttgtata cttatctgta gtctcctc       718

<210> 19
<211> 1198
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (7)
<223> n equals a,t,g, or c

<400> 19
ttatagnaag gtacgcctgc aggtaccggt ccggaattcc cgggtcgacc cacgcgtccg      60
ggctgaagtc ctgcgagcga cgcgcggcgg ggcggcgaga ggaaacgcgg cgccgggccg     120
ggccctggag atggtccccg gcgccgcggg ctggtgttgt ctcgtgctct ggctccccgc     180
gtgcgtcgcg gcccacggct tccgtatcca tgattatttg tactttcaag tgctgagtcc     240
```

```
tggggacatt cgatacatct tcacagccac acctgccaag gactttggtg gtatctttca     300
cacaaggtat gagcagattc accttgtccc cgctgaacct ccagaggcct gcggggaact     360
cagcaacggt ttcttcatcc aggaccagat tgctctggtg gagaggggg gctgctcctt      420
cctctccaag actcgggtgg tccaggagca cggcgggcgg gcggtgatca tctctgacaa     480
cgcagttgac aatgacagct tctacgtgga gatgatccag gacagtaccc agcgcacagc     540
tgacatcccc gccctcttcc tgctcggccg agacggctac atgatccgcc gctctctgga     600
acagcatggg ctgccatggg ccatcatttc catcccagtc aatgtcacca gcatccccac     660
ctttgagctg ctgcaaccgc cctggacctt ctggtagaag agtttgtccc acattccagc     720
cataagtgac tctgagctgg gaaggggaaa cccaggaatt ttgctacttg gaatttggag     780
atagcatctg ggacaagtg gagccaggta gaggaaaagg gtttgggcgt tgctaggctg      840
aaagggaagc cacaccactg gccttccctt ccccagggcc cccaagggtg tctcatgcta     900
caagaagagg caagagacag gccccagggc ttctggctag aacccgaaac aaaaggagct     960
gaaggcaggt ggcctgagag ccatctgtga cctgtcacac tcacctggct ccagcctccc    1020
ctacccaggg tctctgcaca gtgaccttca cagcagttgt tggagtggtt taaagagctg    1080
gtgtttgggg actcaataaa ccctcactga ctttttagca ataaagcttc tcatcagggt    1140
taaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaagg gcggccgc      1198
```

<210> 20
<211> 1033
<212> DNA
<213> Homo sapiens

<400> 20

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ggcacgagct caagatggat gcagagtact ctgggaatga gttccccagg tcagaaggag      60
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caggtgagct gggacaagat gggagactgc tgtcctccac cctctccctc agtagtaaca     180
ggtccttggg ccagcgccag aactctccgc tgccctttca atggagaatc acacacagct     240
tccgctggat ggcccaggtg ttggcctctg agctcagcct ggttgccttt atcctactat     300
tggtcatggc cttctccaag aaatggctgg acctctctag gagcctcttc taccagcgct     360
ggcccgtgga tgtcagcaac agaatccaca catcagccca cgttatgtcc atggggctcc     420
tgcacttttg caaatccagg agctgttctg acttagagaa tgggaaagtc accttcatct     480
tctccaccct catgctattc cccattaaca tctggatctt cgagttggaa aggaatgtat     540
ccatccccat aggctggagc tatttcattg gttggctggt gcttatccta tacttcacct     600
gcgcgatcct ttgctacttc aaccataaaa gtttctggag tctgattctg agccacccca     660
gtggtgccgt gtccygcagc agcagtttcg gctcagtaga agaatctcca agggcacaga     720
cgatcacaga cacccccatc acccaggagg gagtcctgga tcctgagcag aaggatacac     780
atgtgtaatc ttttctgaac tcctggcacc aagttctgtc cattcatctg accccatctc     840
ctcatcctcc cccagccctt gaataggttg gtcctcatca ttgcaaggaa tgagaaaggg     900
aggattttgc actcctctgc tttctccctg ccttgattga gcttgagtga tgtggaataa     960
attgtccgtc tcttctttct caaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa    1020
aaaaaaaaaa aaa                                                       1033
```

<210> 21
<211> 1732
<212> DNA
<213> Homo sapiens

<400> 21

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ggcacgagca gaggtgatcc tgtgcaccaa ggaggtgtcg gtgggcgcac ggaagaacgc      60
ttttgcactg ctcgtggaga tgggccatgc tttcctaagg tttggctcga accaggaaga     120
ggccctgcag tgctacctcg tcctgatcta ccctggcctg gtgggcgcgg tgaccatggt     180
cagctgcagc atcctggccc tgacccacct ccttttcgag tttaaaggtc tgatggggac     240
cagtacagtg gagcagctgc tggagaatgt gtgcctgctt ctggcctccc gcacccgtga     300
cgtggtcaag tctgcactgg gcttcatcaa ggtggcagtg actgtcatgg acgtggcgca     360
cctggccaaa catgtgcagc tggtgatgga agccattggg aagctttcag atgacatgcg     420
gcggcacttc cgcatgaagc ttcggaacct gttcaccaag ttcatccgca agtttggatt     480
tgagctggtg aaaaggctgt tgcccgagga gtaccacaga gtcctggtca acatccggaa     540
agctgaggcc cgggccaaga ggcaccgagc cctgagccag gctgccgtgg aggaggaaga     600
agaggaggag gaggaggagg agcccgccca gggcaaaggt gacagcattg aggagatttt     660
agctgactca gaggacgagg aggacaatga ggaggaggaa agaagccgag gcaaggagca     720
gcggaagctg gcacgacaga ggagccgggc atggctgaaa gagggcggtg gggacgagcc     780
cctcaacttc ctggatccca aggtggccca acgagtcctg gccacgcagc cagggccagc     840
cgggcaggaa gaaggaccac agcttcaagg tgagcgccga tggccggctg atcataaggg     900
aggaggcaga cggcaacaag atggaggaag aggaaggtgc caaaggcgaa gatgaagaga     960
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tggctgaccc aatggaagat gtgatcatca ggaataaaaa gcaccagaag ctcaagcacc   1020
agaaagaggc tgaggaggag gagctggaga taccccctca gtaccaagct ggaggctctg   1080
gcattcatcg ccctgtggcc aagaaggcta tgcctggggc tgaatacaag gccaagaaag   1140
caaaaggtga tgtgaagaag aaaggccggc cggatcccta tgcctacatc cccctcaaca   1200
gaagcaagct caaccgcagg aagaagatga agctgcaggg acagttcaaa ggcctggtga   1260
aggctgcccg gcgaggttcc caggtgggac acaaaaaccg cagaaaggat cgtcgaccct   1320
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tgccttgcca ccagctccag gtgctcaaga ttctggcaga gcctggactc aggatgactt   1440
ggaactaggg cttggctctc agaagtcctg gattttggaa actccaaatg gaatcaccct   1500
tcagagacat ccctggtgcc tggagatggg aatgtggcct cagtgcctct gagtaggtgc   1560
catgaggcac ctttgctttc tgcccagagt ggccatgagc accagaacag atgatctcca   1620
tttccgccag ctgcctgtag ccacgtggca tcctgcctgt ggtctgggtg agatttactg   1680
tgaccagatg tagaataaat gtgtctcatc ctgcaaaaaa aaaaaaaaaa aa           1732
```

```
<210> 22
<211> 840
<212> DNA
<213> Homo sapiens

<400> 22
gtcctaatgg ctcctctcct cccatccctc cctcttcacc tccacacctc cctctgtctc     60
cgcctgtgtc tctctctgtc tctctcagcc tggctctctt ggtctcttcc tctctgcgtc    120
tcactctctg cctcctaccc tgcgtggcgg cttctccccc agctccacgg ccgctctcgg    180
gagcagcgct acaccaagct agccgactgg cagtacatcg aggagtgcgt gcaggccgcc    240
agccccatgc ccctgttcgg aaatggggac atcttgtcat ttgaggatgc caaccgcgcc    300
atgcagactg gtgtcaccgg gatcatgatt gcccgtggcg ccctgctcaa gccgtggctc    360
ttcacggaga tcaaggagca gcggcactgg gacatctcgt cgtccgagcg cctggacatc    420
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aagacccggc gctttctgct cgagtggctg tccttcctgt gccggtacgt gcccgtgggg    540
ctgctggagc ggctcccaca gaggatcaac gagcggccgc cctactacct gggccgcgac    600
tacctggaga cgctgatggc cagccagaag gcagccgact ggatccgcat cagcgagatg    660
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aagtagcctc aggctttccc aggggcaccc tggggcgagg agagtacaat aaattttatt    780
cttttaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa    840
```

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<210> 23
<211> 940
<212> DNA
<213> Homo sapiens

<400> 23
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gggctgcagg aattcggcac gagggctgaa gacacaggcc aggcggaatg aagatgatgg    120
tggtcttgct catgctgtcc tcgctcagcc ggctcctggg cctcatgagg ccatcatctc    180
tcaggcaata cctggactct gtgcccttgc caccctgcca ggagcaacag ccaaaggcta    240
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tgcagttgga ccgccacatc agcacgcaga tccgggagag cccccaggcc atgcaccgca    420
ccatgctcaa ggacctggct acccagacct acatccgttg gcaggagctg ctgacccact    480
gccagcccca ggcccagtat ttcagcccct ggaaagacat ctaaagggac agggtcaggg    540
cagcccaggg ctyctggctt cagcaggaag tgaacaggct cagggaactg gaggaagcga    600
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gccaccgccc cgacccctct cctctctgca agagcctgcc tctgccaccg ccccgacccc    720
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ccatctcctc ttccctaagg cctctgtctc catagctctg gtttccctgg gcctcagtcc    840
tccccaccct ccttcctctg tctccctgtc actaatgtga ggtttctttg tgcacattaa    900
agtcttcttt cagcawmaaa aaaaaaaaaa aaactcgagg                          940
```

```
<210> 24
<211> 801
<212> DNA
<213> Homo sapiens
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<400> 24
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tgggtgggca ggtccatgcc tcccctggcc ccccagctct gcagggcagt gttcctggtt     180
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gtggcctcct cagtgggtga gatgtggcag gtggtggaca tggcccagca ggaagaagac     360
cagtcgtcca agacggcagc tgttcacaag cactctttcc acctcagctt ctgctttagt     420
ctggccagtg tcatggtttt ctcaggaggg ccattgaggc ggacattccc aaatatccaa     480
ctctgcttca tgctcactca ctgaccctcc ctccctcctg ggctccaggt cacaactccc     540
aaaggagatg caggcatggc tctctgcctc tgatcaccat cactgtatct caaggttcag     600
cagcagagat accagttgcc atcagtgcta actgactgcc tctccaggtt cggagtttca     660
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ttcaggacag cagattagag gcaggaggca atgacaataa aataacgata aaatcctgaa     780
aacaaaaaaa aaaaaaaaaa a                                                801

<210> 25
<211> 1969
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (996)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1040)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1058)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1068)
<223> n equals a,t,g, or c

<400> 25
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cgttctccgg acgcaggaac tggtgctcct atgtggtgac ccgcaccatc tcatgccatg     180
tgcagaatgg cacctacctt cagcgagtgc tgcagaactg cccctggccc atgagctgtc     240
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ccgcccgtga gtggaggtgc tgccctgggc actcaggagt gagctgcgag gaagttgcag     360
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tctgtgaagt gggggaggc aggccttcaa ggagggatag aggtacaagg cttcgtctca      1560
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```

<210> 26
<211> 1364
<212> DNA
<213> Homo sapiens

<400> 26

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tgaaggccgc agctcctgct caagctattt tggcagtctg agagaacagt acattctgaa       180
ccacattgac gcagggagca tgggtatctg gacctcaggc actgatatct tcctaagtct       240
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ctccaagcat gcacgatgtt ttattcttct tgtctttctc tcttgtggcc tgcgtgaagg       480
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tgatgaaagg gagagacatc aacagaggcc ctgtgtgctc ccgctgaata aggaggaaag      1020
gaggaagtat gtcatcatcc cgactttctg gccgactcct aaagaaagga aaaacctggg      1080
gctgtttttc ctcccccatac ttatccatct ctgcatctgg gtgctgwttg cagctgtaga     1140
ttatctgctg tatcggctca ttttctcagt gagcaagcag tttcaaagct tgccagggtt      1200
tgaggttcac ttgaaactgc acggagagaa acaaggaact caagatatta tccatgattc      1260
ttcctttaat atatctgtgt ttgaacccaa ctgtatccca aaaccctggc aagctttgaa      1320
actgcttgct cactgagaaa atgagccgat acagcagata atct                       1364
```

<210> 27
<211> 2371
<212> DNA
<213> Homo sapiens

<400> 27

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ttgcgctgct gaacaacatc atcgagatcc gcctggacgc caaaaagttt gtcactgagc       180
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tcccggacat ccccaaggac atcagccagc agatccacaa ggagaaggtg ctcatggtgg       660
agctgttcat gcgggaggag caagacaagc agcagctgct ggaaacctgg atggagaagg       720
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cccgctccca ccagggcccg gtgggtcctg ggttttctgc aaacatggag gaccactttc       960
tgataggaca ttttcctttc ttctttctgt ttctttccc ttgtttttgc acaaagccat      1020
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<210> 28
<211> 867
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (862)
<223> n equals a,t,g, or c

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tctgctctct acggtttttc aagtcagagg caattgatgc ttgtctaatg catccacaca    240
ctgcatgtct gactggcgat gccacgctcc taagtagttc tgccatgaaa cataaaagac    300
aaaggaaaag ccgttacaca tcacacagag aacattttcg ggtcccacag cggtggtggc    360
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<210> 29
<211> 1605
<212> DNA
<213> Homo sapiens

<400> 29
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gggctttgct ggctccagaa agggtgtcct gggccgggtc tatgagacag tggtgatgtt    180
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tcgcttaagc ccagaaggtc aaggttgcag tgagctgaaa tcgcaccact gcactccagc   1560
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<211> 1334
<212> DNA
<213> Homo sapiens

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ggtggccagg cctccaggag gcaccaggca ggccctgtat caggctagga cgctctgagc    300
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tttatataca ctgtttcctg gccccagagc tcatttgggt tcaggcgcac ttcaaaaccc    420
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aaaaaaaaaa aaaa                                                      1334

<210> 31
<211> 1011
<212> DNA
<213> Homo sapiens

<400> 31
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ccccggcctc tactgccctg gctgcagtca gtgcccaggg cgaggaatgt ggccagggga    180
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tcgagccgtc cagctgcggt gggatctgag tatatctagg gcgggtgggc gggtagcagt    360
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gggtgagctg agggtttttg caaggaaaaa aaaaaaaaaa aaaaaaaaaa a 1011

<210> 32
<211> 1308
<212> DNA
<213> Homo sapiens

<400> 32
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gcactgtcct actgtgcctg atgaaaaaaa tcattatgag aaaagttctg gttcttcaga 120
aggtcaaagc aaaacagaat ctgatttttc caacctagac tctgaaaaac acaaaaaagg 180
acctatggag actggattgt ttcctggtag caatgccact ttcaggatac tagaggttgg 240
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<210> 33
<211> 1434
<212> DNA
<213> Homo sapiens

<400> 33
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gcaagtgctc caaggacctg ccccacctag tgttgggggt ggcccgtgcc gaggcacatg 180
acatggcaca catgctggga gcaggagtca ggccaggact gcagatgtgg gtaccatgtg 240
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<210> 34
<211> 2184
<212> DNA
<213> Homo sapiens

<400> 34

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gttcatgtct ctggagcctt caaccagctc ttttaacatc ttgctcttcc cagcattcct     180
tagggtcttt ggctgggctc tgggatggat gccatgggag tacctttact taagctctaa     240
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<210> 35
<211> 1296
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (1215)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1295)

<223> n equals a,t,g, or c

<400> 35
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<210> 36
<211> 1298
<212> DNA
<213> Homo sapiens

<400> 36
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<212> DNA
<213> Homo sapiens

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<211> 3095
<212> DNA
<213> Homo sapiens

<400> 41

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<211> 2320

<212> DNA
<213> Homo sapiens

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<211> 2407
<212> DNA
<213> Homo sapiens

<400> 43
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<210> 44
<211> 1930
<212> DNA
<213> Homo sapiens

<400> 44

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aaaaactcga 1930

<210> 45
<211> 1459
<212> DNA
<213> Homo sapiens

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<210> 46
<211> 1003
<212> DNA
<213> Homo sapiens

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<222> (6)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (28)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (810)
<223> n equals a,t,g, or c

<400> 46
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cgagcacacg tgtgtgcatg ggtcaaaggg gtgtgttcct tctcatccta gatgccttct 240
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tatcagacag tcctcccctg accctccccc ttgtagatat caattcccaa acagagccaa 480
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cccgcccact ctgtatatat gtaagttaaa cccgggcagg ggctgtggcc gtctttgtac      720
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<210> 47
<211> 1358
<212> DNA
<213> Homo sapiens

<400> 47

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<210> 48
<211> 2609
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (2597)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (2603)
<223> n equals a,t,g, or c

<400> 48

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<210> 49
<211> 1898
<212> DNA
<213> Homo sapiens

<400> 49

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<210> 50
<211> 1808
<212> DNA
<213> Homo sapiens

<400> 50

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<210> 51
<211> 955
<212> DNA
<213> Homo sapiens

<400> 51

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<210> 52
<211> 1847
<212> DNA
<213> Homo sapiens

<400> 52
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<210> 53
<211> 2163
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (8)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (15)
<223> n equals a,t,g, or c

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aaa                                                                  2163
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<210> 54
<211> 748
<212> DNA
<213> Homo sapiens

<400> 54

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<210> 55
<211> 1198
<212> DNA
<213> Homo sapiens

<400> 55

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ataaagcatt attttaggtt ttctctttgc cagtttttaa gcagttataa ccatgtaaat     180
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caagatgtga ggacatctat atgaagtata gtaaagaagt ggtgtcagca gatcaatatg      240
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<210> 56
<211> 967
<212> DNA
<213> Homo sapiens

<400> 56
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aattggtgaa agaagaagag agtgagatgg gacccaggtg ggcctggagg tgggatcctg      240
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tttattttaa atattggtga ttctttaacc aggaatgcaa atgctactga agtgctgtgt      900
gtgtctctgt gagagccttc atataaataa attggaattc gatatcaagc ttatcgatac      960
cgtcgac                                                                967


<210> 57
<211> 1147
<212> DNA
<213> Homo sapiens

<400> 57
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agtcaccttt gagctagggg ttattatcct cattttagag atgacaaaat tgaggctcac      180
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<210> 58
<211> 975
<212> DNA
<213> Homo sapiens

<400> 58

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<210> 59
<211> 2733
<212> DNA
<213> Homo sapiens

<400> 59

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<211> 1668
<212> DNA
<213> Homo sapiens

<400> 60

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<211> 1021
<212> DNA
<213> Homo sapiens

<400> 61

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<211> 913
<212> DNA
<213> Homo sapiens

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<211> 1517
<212> DNA
<213> Homo sapiens

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<211> 2751
<212> DNA
<213> Homo sapiens

<400> 64

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<211> 2150
<212> DNA
<213> Homo sapiens

<400> 65
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<211> 1161
<212> DNA
<213> Homo sapiens

<400> 66
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<211> 734
<212> DNA
<213> Homo sapiens

<400> 67
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<210> 68
<211> 1583
<212> DNA
<213> Homo sapiens

<400> 68
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<210> 69
<211> 1444
<212> DNA
<213> Homo sapiens

<400> 69
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aaaa                                                                 1444
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<210> 70
<211> 1892
<212> DNA
<213> Homo sapiens

<400> 70

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cctgctcaag ctattttggc agtctgagag aacagtacat tctgaaccac attgacgcag     180
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ttattaaaga actctttctg aaaaaaaaaa aa                                  1892
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<210> 71
<211> 1439

<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (1281)
<223> n equals a,t,g, or c

<400> 71
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atggatggac tttatccagc atttgggagt ttgctgtttg gttgctctta tttcagtggg     180
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ctttctctct tgtggcctgc gtgaaggcag gaatgctttg attgcagctg gsacagggat     360
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tgaggcaatt cagtggattt atggccttgc cactccacta agtgtatytg atgaccttgt     540
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gcccccacaa tggtctcttt tctccctgct cccttattaa agaactcttt ctgaaaccg     1439
```

<210> 72
<211> 1395
<212> DNA
<213> Homo sapiens

<400> 72
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ttctccagat gatgtccctg gagggggcag gaggtacctc cctctccctc tcctggggca     180
tttgagtctg tggctttggg gtgtcctggg ctccccatct ccttctggcc catctgcctg     240
ctgccctgag ccccggttct gtcagggtcc ctaagggagg acactcaggg cctgtggcca     300
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<210> 73
<211> 1293
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (1214)
<223> n equals a,t,g, or c

<400> 73
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<210> 74
<211> 3147
<212> DNA
<213> Homo sapiens

<400> 74
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<210> 75
<211> 1989
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (31)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (161)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (162)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1702)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1943)
<223> n equals a,t,g, or c

<400> 75
ttaaatgaaa tcaaaattgg ccatttgaca naagttggtt tttccccttt ctgcattttt 60
aggacctcaa agtaatgttt atccagaaac tgctatcatt accagggatt cattcgtgta 120
tttaacaaca tggggcatac attttggcca aatttgaaaa nntcttaaca tacaccccaa 180
aatccctgcc ccaaatttaa gaactagggt ggacacagtg cgtttttcca tgtcgcatct 240
```

```
tctgtgatgg ggctacgata cgtgggagca gagaatgggg aggttggagc gcatgccaga    300
tgaggatcta tcagcaatgg gacgggkcct ccactttagc atctcyaccc tgctcctytc    360
agaggaccgc ctttcattgc attcagctgt gatggtagca cgaacacagg tgcaccgagg    420
acgaggagag caggagcctt gtgctctctc tgcatctgag gcaggacagc acagggtayg    480
gagcagtctg cagagaggcc agctcatcag ggaagcactt gtcttccacc ttgggctttg    540
actgagcact gggcaattgg mcyctgggga tcaaygaaat aatcctaarc agagttactc    600
tatgtcacac tatggaatgt tccaagtasr tggccgtgtt ttcaaaagat rtattttctc    660
cttttgttgt tgccatttca taggtttagg attgggtgtg tgtktctcct ctctgaatgg    720
cactcraatg tttgctgact cctactctgt gtgactgggg tgtacagcta tggactgatg    780
catcccatcc catcatcttt catgatcaaa gcagtctctt ctttttgac agctgaagaa    840
gcatcggtag ggaatccaga aggagcgttc atgaaggtgt tacaagcccg gaagaactam    900
acaagcactg agctgattgt tgagccagag gagccctcag acagcagtgg catcaacttg    960
tcaggctttg ggagtgagca gctagacacc aatgacgaga gtgatkttat cagtacacta   1020
agttacatct tgccwtattt ctcagcrgta aacctagatg tgraatcamt gttactaccg   1080
ttcattaaac tgccaaccmc aggaaacagc ctggcaaaga ttcaaactgt aggccaaaac   1140
crgcararag tgaakagagt cctcatgggc ccaaggagca tccagaaaag gcacttcaaa   1200
gaggtrggaa ggcagagcat caggagggaa cagggtgccc aggcatctgt ggagaacgct   1260
gccgaagaaa aaaggctcgg gagtccagcc ccaagggags tggaacagcc ycacacacag   1320
caggggcctg agaagttagc gggaaacgcc rtctacacca agccttcstt cacccaagag   1380
cataaggcag cagtctctgt gctgamaccc ttctccaagg gcgcgccttc tacctccagc   1440
cctgcaaaag ccctaccaca ggtgagagac agatggaaag acwwmacmca crctatttcc   1500
attttagaaa gtgcaaaggc tagagttaca aatatgaagg cttctaaacc aatttcacat   1560
tccagaaaaa aataccgctt tcacaaaact cgctcccgca tgacccacag aacacccaag   1620
gtcaaaaaga gtccaaagtt cagaaagaaa agttatctga gtagactgat gctcgcaaac   1680
aggcctccgt tctctgcagc gnagagcctc ataaattccc cttcacaagg ggcttttca    1740
tccttaggag acctgagtcc tcaagaaaac ccttttytgg ragtatctgc tccttcagaa   1800
cattttatag aaaccactaa tataaaagac acaactgcaa gaaatgcctt ggaagaaaat   1860
gtttttatgg aaaacactaa catgccagaa gtcaccatct ctgaaaacac aaactacaat   1920
catcctcctg aggcagattc cgntgggact gcattcaact tagggccaac tgttaaacaa   1980
actgagaca                                                           1989
```

<210> 76
<211> 1879
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (41)
<223> n equals a,t,g, or c

<400> 76

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agacctttga taacataacc attagcagag aggctcaggg ngaggtccct gcctcggact     60
caaagaccga atgcacggcc ttgtagggga cgccccagat tgtcagggat kgggggatgg    120
tccttggagt tttgcatgct ctcctccctc ccacttctgc accctttcac cacctcgagg    180
agatttgctc cccattagcg aatgaaattg atgcagtcct acctaactcg attccctttg    240
gcttggtggg taggcctgca gggcactttt attccaaccc ctggtcaytc agtamtstkt    300
tactccagga aggcacagga tggtacctaa agagaattag agaatgaacc tggckrgacg    360
gatgtctaat cctgcrccta gctgggttgg tcagtagaac ctattttcag actcaaaaac    420
catcttcaga aagaaaaggc ccagggaagg aatgtatgag aggctctccc agatgaggaa    480
gtgtactctc tatgactatc aagctcaggc ctctcccttt ttttaaacca aagtctggca    540
accaagagca gcagctccat ggcctccttg ccccagatca gcctgggtca ggggacatag    600
tgtcattgtt tggaaactgc agacacaagg tgtgggtcta tcccacttcc tagtgctccc    660
cacattcccc atcagggctt cctcacgtgg amaggtktgc tartccaggc agttcacttg    720
cagtttcctt gtcctcatgc ytcggggatg ggagccmcgm cygaactaga gttcaggctg    780
gatacatgtg ctcacctgct gctcttgtct tcctaagaga cagagagtgg ggcagatgga    840
ggagaagaaa gtgaggaatg agtagcatag cattctgcca aaagggcccc agattcttaa    900
tttagcaaac taagaagccc aattcaaaag cattgtggct aaagtctaac gctcctctct    960
tggtcagata acaaaagccc tccctgttgg atcttttgaa ataaaacgtg caagttatcc   1020
aggctcgtag cctgcatgct gccaccttga atcccaggga gtatctgcac ctggaatagc   1080
tctccacccc tctctgcctc cttactttct gtgcaagatg acttcctggg ttaacttcct   1140
tctttccatc cacccaccca ctggaatctc tttccaaaca tttttccatt ttcccacaga   1200
tgggctttga ttagctgtcc tctctccatg cctgcaaagc tccagatttt tggggaaagc   1260
tgtacccaac tggactgccc agtgaactgg gatcattgag tacagtcgag cacacgtgtg   1320
tgmatgggtc aaaggggtgt gttccttctc atcctagatg ccttctctgt gccttccaca   1380
```

```
gcctcctgcc tgattacacc actgcccccg ccccaccctc agccatccca attcttcctg   1440
gccagtgcgc tccagcctta tctaggaaag gargagtggg tgtagccgtg cagcaagatt   1500
ggggcctccc ccatcccagc ttctccacca tcccagcaag tcaggatatc agacartcct   1560
cccctgaccc tccccccttgt agatatcaat tcccaaacag agccaaatac tctatatcta   1620
tagtcacagc cctgtacagc atttttcata agttatatag taaatggtct gcatgatttg   1680
tgcttctagt gctctcattt ggaaatgagg caggcttctt ctatgaaatg taaagaaaga   1740
aaccactttg tatattttgt aataccacct ctgtggccat gcctgccccg cccactctgt   1800
atatatgtaa gttaaacccg ggcaggggct gtggccgtct ttgtactctg gtgatttta    1860
aaaattgaat ctttgtact                                                1879
```

<210> 77
<211> 1879
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (41)
<223> n equals a,t,g, or c

<400> 77

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agacctttga taacataacc attagcagag aggctcaggg ngaggtccct gcctcggact     60
caaagaccga atgcacggcc ttgtagggga cgccccagat tgtcagggat kgggggatgg    120
tccttggagt tttgcatgct ctcctccctc ccacttctgc accctttcac cacctcgagg    180
agatttgctc cccattagcg aatgaaattg atgcagtcct acctaactcg attccctttg    240
gcttggtggg taggcctgca gggcactttt attccaaccc ctggtcaytc agtamtstkt    300
tactccagga aggcacagga tggtacctaa agagaattag agaatgaacc tggckrgacg    360
gatgtctaat cctgcrccta gctgggttgg tcagtagaac ctattttcag actcaaaaac    420
catcttcaga aagaaaaggc ccagggaagg aatgtatgag aggctctccc agatgaggaa    480
gtgtactctc tatgactatc aagctcaggc ctctcccttt ttttaaacca aagtctggca    540
accaagagca gcagctccat ggcctccttg ccccagatca gcctgggtca ggggacatag    600
tgtcattgtt tggaaactgc agacacaagg tgtgggtcta tcccacttcc tagtgctccc    660
cacattcccc atcagggctt cctcacgtgg amaggtktgc tartccaggc agttcacttg    720
cagtttcctt gtcctcatgc ytcggggatg ggagccmcgm cygaactaga gttcaggctg    780
gatacatgtg ctcacctgct gctcttgtct tcctaagaga cagagagtgg ggcagatgga    840
ggagaagaaa gtgaggaatg agtagcatag cattctgcca aaagggcccc agattcttaa    900
tttagcaaac taagaagccc aattcaaaag cattgtggct aaagtctaac gctcctctct    960
tggtcagata acaaaagccc tccctgttgg atcttttgaa ataaaacgtg caagttatcc   1020
aggctcgtag cctgcatgct gccaccttga atcccaggga gtatctgcac ctggaatagc   1080
tctccacccc tctctgcctc cttactttct gtgcaagatg acttcctggg ttaacttcct   1140
tctttccatc cacccaccca ctggaatctc tttccaaaca tttttccatt ttcccacaga   1200
tgggctttga ttagctgtcc tctctccatg cctgcaaagc tccagatttt tggggaaagc   1260
tgtacccaac tggactgccc agtgaactgg gatcattgag tacagtcgag cacacgtgtg   1320
tgcatgggtc aaaggggtgt gttccttctc atcctagatg ccttctctgt gccttccaca   1380
gcctcctgcc tgattacacc actgcccccg ccccaccctc agccatccca attcttcctg   1440
gccagtgcgc tccagcctta tctaggaaag gargagtggg tgtagccgtg cagcaagatt   1500
ggggcctccc ccatcccagc ttctccacca tcccagcaag tcaggatatc agacartcct   1560
cccctgaccc tccccccttgt agatatcaat tcccaaacag agccaaatac tctatatcta   1620
tagtcacagc cctgtacagc atttttcata agttatatag taaatggtct gcatgatttg   1680
tgcttctagt gctctcattt ggaaatgagg caggcttctt ctatgaaatg taaagaaaga   1740
aaccactttg tatattttgt aataccacct ctgtggccat gcctgccccg cccactctgt   1800
atatatgtaa gttaaacccg ggyaggggct gtggccgtct ttgtactctg gtgatttta    1860
aaaattgaat ctttgtact                                                1879
```

<210> 78
<211> 955
<212> DNA
<213> Homo sapiens

<400> 78

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ccacgcgtcc gggggactct gcaataaggc agtgaaagcc ctagcccagt gcctggcagc     60
tgctgtgatg atgattatta ttattaatgt cacccccgct ccccgccacg cacacatggg    120
ctagaggggt gacttccaca cccctggctt agactgtcct gcaggctggg tgttttcttt    180
gtgattccca aggccccaag cccagtggga aatttccgcc acttccatgt gccctgccac    240
```

```
gacttcctgc catctgctga ctcggtgtga catgacacac ggctgcctct ccctggccag     300
catggccgcg gggcttgggt ctgtctcact gttcttgttt gttcaacagt ggactccaac     360
gacagcctct acgggggaga ctccaagttc ctggcagaaa acaacaagct gtgtgagacg     420
gtgatggctc agatcctaga gcatctgaaa accctggcca aggacgaggc cctgaagcgc     480
cagagctcgt tgggcctttc cttctttaac agcatcttgg cccatgggga cctacgcaac     540
aacaagctca accagctctc cgtcaacctg tggcacctgg cacagaggca cggctgtgca     600
gacaccagga ccatggtgaa aacgctagaa tacatcaaga agcaaagcaa acaaccagac     660
atgactcatc tgacggagct ggccctcaga ctccctctgc aaacaaggac ctgacccccg     720
ggcccatccc caggctcagg gactctggtg ccaaatccag aaagatctgc tctgctgccc     780
tgaactctta cggcaattta ggtttctcat ttttctttc tttttacata tgtacaaatt     840
gttttaagct ttggcctcta tccaggttat tctgacaatg aagaaatggg agttgtcaga     900
gcattaaaat gcaatcttca ctaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa          955
```

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<210> 79
<211> 2309
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (2119)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (2138)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (2145)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (2187)
<223> n equals a,t,g, or c

<400> 79
ggctgctgca ggcgtccggc ttggacgaac cgccgttccc agtgctggga ccctttaagt       60
atgcaggtga tagactagag aacaagacct ctgtctccgt agcatcctgg agcagtctga      120
atgccagaat ggataaccgt tttgctacag catttgtaat tgcttgtgtg cttagcctca      180
tttccaccat ctacatggca gcctccattg gcacagactt ctggtatgaa tatcgaagtc      240
cagttcaaga aaattccagt gatttgaata aaagcatctg ggatgaattc attagtgatg      300
aggcagatga aaagacttat aatgatgcac tttttcgata caatggcaca gtgggattgt      360
ggagacggtg tatcaccata cccaaaaaca tgcattggta tagcccacca gaaaggacag      420
agtcatttga tgtggtcaca aaatgtgtga gtttcacact aactgagcag ttcatggaga      480
aatttgttga tcccggaaac cacaatagcg ggattgatct ccttaggacc tatctttggc      540
gttgccagtt ccttttacct tttgtgagtt taggtttgat gtgctttggg gctttgatcg      600
gactttgtgc ttgcatttgc cgaagcttat atcccaccat tgccacgggc attctccatc      660
tccttgcagg tctgtgtaca ctgggctcag taagttgtta tgttgctgga attgaactac      720
tccaccagaa actagagctc cctgacaatg tatccggtga atttggatgg tccttctgcc      780
tggcttgtgt ctctgctccc ttacagttca tggcttctgc tctcttcatc tgggctgctc      840
acaccaaccg gaaagagtac accttaatga aggcatatcg tgtggcatga gcaagaaact      900
gcctgcttta caattgccat tttatttt ttaaaataat actgatattt tccccacctc       960
tcaattgttt ttaattttta tttgtggata taccatttta ttatgaaaat ctattttatt     1020
tatacacatt caccactaaa tacacactta ataccactaa aatttatgtg gtttacttta     1080
agcgatgcca tctttcaaat aaactaatct aggtctagac agaaagaaat ggatagagac     1140
ttgacacaaa tttatgaaag aaaattggga gtaggaatgt gaccgaaaac aagttgtgct     1200
aatgtctgtt agacttttca gtaaaactaa agtaactgta tctgttcaac taaaaactct     1260
atattagttt ctttgggaaa cctctcatcg tcaaaacttt atgttcactt tgctgttgta     1320
gatagccagt caaccagcag tattagtgct gttttcaaag atttaagctc tataaaattg     1380
ggaaattatc taagatcatt ttccctaagc attgacacat agcttcatct gaggtgagat     1440
atggcagctg tttgtatctg cactgtgtct gtctacaaaa agtgaaaaat acagtgttta     1500
cttgaaattt taactttgta actgcaagaa ttccagttca gccgggcgag gattagtatt     1560
```

```
atttttaact ctccgtaaga ttttcagtac caccaaattg ttttggattt tttttctttc      1620
ctcttcacat accagggtta ttaaaagtgt gctttctttt tacattatat tacagttaca      1680
aggtaaaatt cctcaactgc tatttattta ttccagccca gtactataaa gaacgtttca      1740
ccataatgac cctccagagc tgggaaacct accacaagat ctaaagttct ggctgtccat      1800
taacctccaa ctatggtctt tatttcttgt ggtaatatga tgtgcctttc cttgcctaaa      1860
tcccttcctg gtgtgtatca acattattta atgtcttcta attcagtcat tttttttataa     1920
gtatgtctat aaacattgaa ctttaaaaaa cttatttatt tattccacta ctgtagcaat      1980
tgacagatta aaaaaatgta acttcataat ttcttaccat aacctcaatg tctttttttaa     2040
aaaataaaat taaaaatgaa aagagaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa      2100
actcgagggg gggcccggna cccaattcgc cctatagnga gtcgnattac aattcactgg      2160
ccgtcgtttt acaacgtcgt gactggnaaa accctggcgt tacccaactt aatcgccttg      2220
cagcacatcc ccctttcgcc agctggcgta atagcgaaga ggcccgcacc gatcgccctt      2280
cccaacagtt gcgcagcctg aatggcgaa                                        2309
```

<210> 80
<211> 2619
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (81)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (2323)
<223> n equals a,t,g, or c

<400> 80

```
cgctgagaag gagcagacaa gatggcgacg tccgtggggc accgatgtct gggattactg        60
cacggggtcg cgccgtggcg nagcagcctc catccctgtg agatcactgc cctgagccaa       120
tccctacagc ccttacggaa gctgcctttt agagcctttc gcacagatgc cagaaaaatc       180
cacactgccc ctgcccgaac catgttcctg ctgcgtcccc tgcccattct gttggtgaca       240
ggcggcgggt atgcagggta ccggcagtat gagaagtaca gggagcgaga gctggagaag       300
ctgggattgg agattccacc caaacttgct ggtcactggg aggtggcttt gtacaagtca       360
gtgccaacgc gcttgctgtc acgggcctgg ggtcgcctca atcaggtgga gctgccacac       420
tggctgcgca ggcccgtcta cagcctgtac atctggacgt ttggggtgaa catgaaagag       480
gccgctgtgg aggacctgca tcactaccgc aacctcagcs agttcytccg gcgcaagctg       540
aaagccckcag gcccggcctg tytgtggcct gcacagcgtg attagcccat cggatggaag      600
gwtcctcaac tttgggcagg tgaagaactg tgaggtggag caggtaaagg gggtcaccta       660
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tgggtcactg cacaggcggg gctaggcagc cctgctgctg tgtggctgga ccgggaggga       780
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gtccccatgc gtaagggcga gcacctgggc gagttcaacc tgggctccac catcgtgctc      1020
atcttcgagg cccccaagga cttcaatttc cagctgaaaa caggacagaa aatccgcttt      1080
ggggaagccc tgggctcgct ctagagtctc tttcctgatt atggctgcta agggatcttt      1140
tccaaacaga gtgagggtct tttcaagagg gaggcccatg aggccatcca ggtaagggcc      1200
tgcctcagcg tggttgggag tctgaccagg taggacttga atgattcggc tmccacctgt      1260
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aaacactgta tagacggaag agcctgcatt cctgaccgaa ccttcagttg gtctcggttg      1440
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ttttttggcc ttaacgctcc tgctgcacag ggtgaggtgc ctccttggca cagactgtgg      1560
atgcctctcc cccagcagag ccacacagcc ttcgtgacaa ctgctttccg ttcccacatt      1620
cacctcatcc tgctctttag aaaaagcagt ctttgtgctt gtggctgaac gcatcacccct     1680
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caatcactga acctgcctca tggcaccaga gtgaacaggg caggcaggta gtaggcccag      1860
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ttgctgctat tgattcaggg tctgtcttgg aggcagagga cccttggtgg atagttggtc      1980
agtgcctgga aaacctgtcc cagtttatca ggaacgcagg cctggggagc cccagtggc       2040
ggggacaggg ccagatttca tgttgaccct ggggatgctg tgaatttctc ctgcaggaga      2100
```

```
gacatcattg aatttttca actgtatcag tagcacagta tttttgtatg aaaagtggga    2160
gacttctgaa cagtaattca tttaattgca aagcattttg aaataaaaaa aatcaaactt   2220
aaaactgtgt tctgttcctg gaggtggcct gaggctcccc gacagcccag cacctgcgtt   2280
cctggtgcga acacgctagc acagggcctg ccgcaggcct ggnatgcggg caacgcaggc   2340
gccaatgggc tggcgctgag cctggagctc ctctgccagc agctggatct gcatttccac   2400
ctgttccagc tctttctgca cctgctgctc tacttcctgg tcctcaggcc ccggctcctc   2460
tctgttcagc tccagccacc tgcgcaggct gctcgcttgc cgccgacaga ccagargttc   2520
tgcttggtgg tctggtartg cagtagttgc tgctggatct cctcaagttc agcctcaagg   2580
tccaggctgc ctgcagttga aagtcccgga cgcgtgggc                          2619
```

<210> 81
<211> 884
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (873)
<223> n equals a,t,g, or c

<400> 81

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gaaaaaacct ggcccgagag tcgaagggcc gccccaggcc aacagaaatc acccggcctt    120
acctctgtcc ccacccttac cttcccccac ataccgcccc ctgcttgggt tcccacccca    180
gcgcttgccg ctgctcccgc tcctgtcccc acagcctcct cctcccattc tccatcacca    240
gggaatgccc cggttcccac agggtccccc agatgcctgt ttttcctcag accatacttt    300
ccagtcggat caattctatt gccattcaga tgtcccctca tcagcccatg caggtttctt    360
cgtcgaagac aattttatgg ttggtcctca gctgcctatg cccttcttcc ccacaccccg    420
ttatcagcgg cctgccccag tggtacatag ggttttggc aggtatcgtc cccgtggccc     480
ctatacgccc tggggacagc ggcctcgacc ttcaaagaga agggccccag ccaatcctga    540
gccaaggcct caatagacgg acctaggcct tatttcctct ttatgaacat ggattggaca    600
gatctgacac ttcctttcca ttgcttggcc tgaacagact gaccttgtta acttaagcct    660
ggagtccatg cctcgtcttc cttttgttca ttgctgttac caagaaagcc aaggaagagc    720
agcctgactc attcttcttg gctgcagcct cttccccact tcctgggagt gacccagcgt    780
tattcctgcc tcctcactcc tattctcttt gcctttgtgt aaaaataaaa tggaaataaa    840
caagttgcac agaaaaaaaa aaaaaaaaaa aancccaagg gggg                     884
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<210> 82
<211> 1086
<212> DNA
<213> Homo sapiens

<400> 82

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ggattctagg acagggatgg gggtgcagca ctgatccagt tgacaacagg aggcagaggc     60
atcatggagg gtccccgggg atggctggtg ctctgtgtgc tggccatatc gctggcctct    120
atggtgaccg aggacttgtg ccgagcacca gacgggaaga aaggggaggc aggaagacct    180
ggcagacggg ggcggccagg cctcaagggg gagcaagggg agccgggggc ccctggcatc    240
cggacaggca tccaaggcct taaaggagac caggggggaac ctgggccctc tggaaacccc   300
ggcaaggtgg gctacccagg gcccagcggc cccctcggag cccgtggcat cccgggaatt    360
aaaggcacca agggcagccc aggaaacatc aaggaccagc cgaggccagc cttctccgcc    420
attcggcgga accccccaat gggggcaac gtggtcatct tcgacacggt catcaccaac     480
caggaagaac cgtaccagaa ccactccggc cgattcgtct gcactgtacc cggctactac    540
tacttcacct tccaggtgct gtcccagtgg gaaatctgcc tgtccatcgt ctcctcctca    600
aggggccagg tccgacgctc cctgggcttc tgtgacacca ccaacaaggg gctcttccag    660
gtggtgtcag gggcatggt gcttcagctg cagcagggtg accaggtctg ggttgaaaaa     720
gaccccaaaa agggtcacat ttaccagggc tctgaggccg acagcgtctt cagcggcttc    780
ctcatcttcc catctgcctg agccagggaa ggaccccctc ccccacccac ctctctggct    840
tccatgctcc gcctgtaaaa tgggggcgct attgcttcag ctgctgaagg gagggggctg    900
gctctgagag ccccaggact ggctgccccg tgacacatgc tctaagaagc tcgtttctta    960
gacctcttcc tggaataaac atctgtgtct gtgtctgctg aaaaaaaaaa aaaaaaaaaa   1020
aaaaaaaaaa aaaaaaaaaa aaaaaactcg aggggggccc cggtacccaa ttcgccgtat   1080
aatgag                                                              1086
```

<210> 83
<211> 563
<212> PRT
<213> Homo sapiens

<400> 83
Met Gly Ser Leu Ser Asn Tyr Ala Leu Leu Gln Leu Thr Leu Thr Ala
1   5   10   15

Phe Leu Thr Ile Leu Val Gln Pro Gln His Leu Leu Ala Pro Val Phe
            20   25   30

Arg Thr Leu Ser Ile Leu Thr Asn Gln Ser Asn Cys Trp Leu Cys Glu
        35   40   45

His Leu Asp Asn Ala Glu Gln Pro Glu Leu Val Phe Val Pro Ala Ser
    50   55   60

Ala Ser Thr Trp Trp Thr Tyr Ser Gly Gln Trp Met Tyr Glu Arg Val
65   70   75   80

Trp Tyr Pro Gln Ala Glu Val Gln Asn His Ser Thr Ser Ser Tyr Arg
                85   90   95

Lys Val Thr Trp His Trp Glu Ala Ser Met Glu Ala Gln Gly Leu Ser
            100   105   110

Phe Ala Gln Val Arg Leu Leu Glu Gly Asn Phe Ser Leu Cys Val Glu
        115   120   125

Asn Lys Asn Gly Ser Gly Pro Phe Leu Gly Asn Ile Pro Lys Gln Tyr
    130   135   140

Cys Asn Gln Ile Leu Trp Phe Asp Ser Thr Asp Gly Thr Phe Met Pro
145   150   155   160

Ser Ile Asp Val Thr Asn Glu Ser Arg Asn Asp Asp Asp Asp Pro Ser
                165   170   175

Val Cys Leu Gly Thr Arg Gln Cys Ser Trp Phe Ala Gly Cys Thr Asn
            180   185   190

Arg Thr Trp Asn Ser Ser Ala Val Pro Leu Ile Gly Leu Pro Asn Thr
        195   200   205

Gln Asp Tyr Lys Trp Val Asp Arg Asn Ser Gly Leu Thr Trp Ser Gly
    210   215   220

Asn Asp Thr Cys Leu Tyr Ser Cys Gln Asn Gln Thr Lys Gly Leu Leu
225   230   235   240

Tyr Gln Leu Phe Arg Asn Leu Phe Cys Ser Tyr Gly Leu Thr Glu Ala
                245   250   255

His Gly Lys Trp Arg Cys Ala Asp Ala Ser Ile Thr Asn Asp Lys Gly
            260   265   270

His Asp Gly His Arg Thr Pro Thr Trp Trp Leu Thr Gly Ser Asn Leu
        275   280   285

Thr Leu Ser Val Asn Asn Ser Gly Leu Phe Phe Leu Cys Gly Asn Gly
    290   295   300

Val Tyr Lys Gly Phe Pro Pro Lys Trp Ser Gly Arg Cys Gly Leu Gly
305   310   315   320

```
Tyr Leu Val Pro Ser Leu Thr Arg Tyr Leu Thr Leu Asn Ala Ser Gln
                325                 330                 335

Ile Thr Asn Leu Arg Ser Phe Ile His Lys Val Thr Pro His Arg Cys
            340                 345                 350

Thr Gln Gly Asp Thr Asp Asn Pro Pro Leu Tyr Cys Asn Pro Lys Asp
        355                 360                 365

Asn Ser Thr Ile Arg Ala Leu Phe Pro Ser Leu Gly Thr Tyr Asp Leu
    370                 375                 380

Glu Lys Ala Ile Leu Asn Ile Ser Lys Ala Met Glu Gln Glu Phe Ser
385                 390                 395                 400

Ala Thr Lys Gln Thr Leu Glu Ala His Gln Ser Lys Val Ser Ser Leu
                405                 410                 415

Ala Ser Ala Ser Arg Lys Asp His Val Leu Asp Ile Pro Thr Thr Gln
            420                 425                 430

Arg Gln Thr Ala Cys Gly Thr Val Gly Lys Gln Cys Cys Leu Tyr Ile
        435                 440                 445

Asn Tyr Ser Glu Glu Ile Lys Ser Asn Ile Gln Arg Leu His Glu Ala
    450                 455                 460

Ser Glu Asn Leu Lys Asn Val Pro Leu Leu Asp Trp Gln Gly Ile Phe
465                 470                 475                 480

Ala Lys Val Gly Asp Trp Phe Arg Ser Trp Gly Tyr Val Leu Leu Ile
                485                 490                 495

Val Leu Phe Cys Leu Phe Ile Phe Val Leu Ile Tyr Val Arg Val Phe
            500                 505                 510

Arg Lys Ser Arg Arg Ser Leu Asn Ser Gln Pro Leu Asn Leu Ala Leu
        515                 520                 525

Ser Pro Gln Gln Ser Ala Gln Leu Leu Val Ser Glu Thr Ser Cys Gln
    530                 535                 540

Val Ser Asn Arg Ala Met Lys Gly Leu Thr Thr His Gln Tyr Asp Thr
545                 550                 555                 560

Ser Leu Leu


&lt;210&gt; 84
&lt;211&gt; 152
&lt;212&gt; PRT
&lt;213&gt; Homo sapiens

&lt;400&gt; 84
Met Gly Val His Val Gly Ala Ala Leu Gly Ala Leu Trp Phe Cys Leu
  1               5                  10                  15

Thr Gly Ala Leu Glu Val Gln Val Pro Glu Asp Pro Val Val Ala Leu
            20                  25                  30

Val Gly Thr Asp Ala Thr Leu Cys Cys Ser Phe Ser Pro Glu Pro Gly
        35                  40                  45

Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys
    50                  55                  60
```

```
Gln Leu Val His Ser Phe Ala Glu Gly Gln Asp Gln Gly Ser Ala Tyr
 65                  70                  75                  80

Ala Asn Arg Thr Ala Leu Phe Leu Asp Leu Leu Ala Gln Gly Asn Ala
                 85                  90                  95

Ser Leu Arg Leu Gln Ser Val Arg Val Ala Asp Glu Gly Gln Leu His
            100                 105                 110

Leu Leu Arg Glu His Pro Gly Phe Arg Gln Arg Cys Arg Gln Pro Ala
        115                 120                 125

Gly Gly Arg Ser Leu Leu Glu Ala Gln His Asp Pro Gly Ala Gln Gln
    130                 135                 140

Gly Pro Ala Ala Arg Gly Thr Trp
145                 150


<210> 85
<211> 215
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (7)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 85
Met Leu Pro Trp Thr Ala Xaa Gly Leu Ala Leu Ser Leu Arg Leu Ala
  1               5                  10                  15

Leu Ala Arg Ser Gly Ala Glu Arg Gly Pro Pro Ala Ser Ala Pro Arg
             20                  25                  30

Gly Asp Leu Met Phe Leu Leu Asp Ser Ser Ala Ser Val Ser His Tyr
         35                  40                  45

Glu Phe Ser Arg Val Arg Glu Phe Val Gly Gln Leu Val Ala Pro Leu
     50                  55                  60

Pro Leu Gly Thr Gly Ala Leu Arg Ala Ser Leu Val His Val Gly Ser
 65                  70                  75                  80

Arg Pro Tyr Thr Glu Phe Pro Phe Gly Gln His Ser Ser Gly Glu Ala
                 85                  90                  95

Ala Gln Asp Ala Val Arg Ala Ser Ala Gln Arg Met Gly Asp Thr His
            100                 105                 110

Thr Gly Leu Ala Leu Val Tyr Ala Lys Glu Gln Leu Phe Ala Glu Ala
        115                 120                 125

Ser Gly Ala Arg Pro Gly Val Pro Lys Val Leu Val Trp Val Thr Asp
    130                 135                 140

Gly Gly Ser Ser Asp Pro Val Gly Pro Pro Met Gln Glu Leu Lys Asp
145                 150                 155                 160

Leu Gly Val Thr Val Phe Ile Val Ser Thr Gly Arg Gly Asn Phe Leu
                165                 170                 175

Glu Leu Ser Ala Ala Ala Ser Ala Pro Ala Glu Lys His Leu His Phe
            180                 185                 190
```

```
Val Asp Val Asp Asp Leu His Ile Ile Val Gln Glu Leu Arg Gly Ser
        195                 200                 205

Ile Leu Asp Ala Met Arg Pro
    210                 215


<210> 86
<211> 831
<212> PRT
<213> Homo sapiens

<400> 86
Met Lys Val His Met His Thr Lys Phe Cys Leu Ile Cys Leu Leu Thr
  1               5                  10                  15

Phe Ile Phe His His Cys Asn His Cys His Glu Glu His Asp His Gly
             20                  25                  30

Pro Glu Ala Leu His Arg Gln His Arg Gly Met Thr Glu Leu Glu Pro
         35                  40                  45

Ser Lys Phe Ser Lys Gln Ala Ala Glu Asn Glu Lys Lys Tyr Tyr Ile
     50                  55                  60

Glu Lys Leu Phe Glu Arg Tyr Gly Glu Asn Gly Arg Leu Ser Phe Phe
 65                  70                  75                  80

Gly Leu Glu Lys Leu Leu Thr Asn Leu Gly Leu Gly Glu Arg Lys Val
                 85                  90                  95

Val Glu Ile Asn His Glu Asp Leu Gly His Asp His Val Ser His Leu
            100                 105                 110

Asp Ile Leu Ala Val Gln Glu Gly Lys His Phe His Ser His Asn His
        115                 120                 125

Gln His Ser His Asn His Leu Asn Ser Glu Asn Gln Thr Val Thr Ser
    130                 135                 140

Val Ser Thr Lys Arg Asn His Lys Cys Asp Pro Glu Lys Glu Thr Val
145                 150                 155                 160

Glu Val Ser Val Lys Ser Asp Asp Lys His Met His Asp His Asn His
                165                 170                 175

Arg Leu Arg His His His Arg Leu His His His Leu Asp His Asn Asn
            180                 185                 190

Thr His His Phe His Asn Asp Ser Ile Thr Pro Ser Glu Arg Gly Glu
        195                 200                 205

Pro Ser Asn Glu Pro Ser Thr Glu Thr Asn Lys Thr Gln Glu Gln Ser
    210                 215                 220

Asp Val Lys Leu Pro Lys Gly Lys Arg Lys Lys Lys Gly Arg Lys Ser
225                 230                 235                 240

Asn Glu Asn Ser Glu Val Ile Thr Pro Gly Phe Pro Pro Asn His Asp
                245                 250                 255

Gln Gly Glu Gln Tyr Glu His Asn Arg Val His Lys Pro Asp Arg Val
            260                 265                 270

His Asn Pro Gly His Ser His Val His Leu Pro Glu Arg Asn Gly His
```

```
        275                 280                 285

Asp Pro Gly Arg Gly His Gln Asp Leu Asp Pro Asp Asn Glu Gly Glu
    290                 295                 300

Leu Arg His Thr Arg Lys Arg Glu Ala Pro His Val Lys Asn Asn Ala
305                 310                 315                 320

Ile Ile Ser Leu Arg Lys Asp Leu Asn Glu Asp Asp His His His Glu
                325                 330                 335

Cys Leu Asn Val Thr Gln Leu Leu Lys Tyr Tyr Gly His Gly Ala Asn
            340                 345                 350

Ser Pro Ile Ser Thr Asp Leu Phe Thr Tyr Leu Cys Pro Ala Leu Leu
        355                 360                 365

Tyr Gln Ile Asp Ser Arg Leu Cys Ile Glu His Phe Asp Lys Leu Leu
    370                 375                 380

Val Glu Asp Ile Asn Lys Asp Lys Asn Leu Val Pro Glu Asp Glu Ala
385                 390                 395                 400

Asn Ile Gly Ala Ser Ala Trp Ile Cys Gly Ile Ile Ser Ile Thr Val
                405                 410                 415

Ile Ser Leu Leu Ser Leu Leu Gly Val Ile Leu Val Pro Ile Ile Asn
            420                 425                 430

Gln Gly Cys Phe Lys Phe Leu Leu Thr Phe Leu Val Ala Leu Ala Val
        435                 440                 445

Gly Thr Met Ser Gly Asp Ala Leu Leu His Leu Leu Pro His Ser Gln
    450                 455                 460

Gly Gly His Asp His Ser His Gln His Ala His Gly His Gly His Ser
465                 470                 475                 480

His Gly His Glu Ser Asn Lys Phe Leu Glu Glu Tyr Asp Ala Val Leu
                485                 490                 495

Lys Gly Leu Val Ala Leu Gly Gly Ile Tyr Leu Leu Phe Ile Ile Glu
            500                 505                 510

His Cys Ile Arg Met Phe Lys His Tyr Lys Gln Gln Arg Gly Lys Gln
        515                 520                 525

Lys Trp Phe Met Lys Gln Asn Thr Glu Glu Ser Thr Ile Gly Arg Lys
    530                 535                 540

Leu Ser Asp His Lys Leu Asn Asn Thr Pro Asp Ser Asp Trp Leu Gln
545                 550                 555                 560

Leu Lys Pro Leu Ala Gly Thr Asp Asp Ser Val Val Ser Glu Asp Arg
                565                 570                 575

Leu Asn Glu Thr Glu Leu Thr Asp Leu Glu Gly Gln Gln Glu Ser Pro
            580                 585                 590

Pro Lys Asn Tyr Leu Cys Ile Glu Glu Glu Lys Ile Ile Asp His Ser
        595                 600                 605

His Ser Asp Gly Leu His Thr Ile His Glu His Asp Leu His Ala Ala
    610                 615                 620

Ala His Asn His His Gly Glu Asn Lys Thr Val Leu Arg Lys His Asn
```

625 630 635 640

His Gln Trp His His Lys His Ser His His Ser His Gly Pro Cys His
645 650 655

Ser Gly Ser Asp Leu Lys Glu Thr Gly Ile Ala Asn Ile Ala Trp Met
660 665 670

Val Ile Met Gly Asp Gly Ile His Asn Phe Ser Asp Gly Leu Ala Ile
675 680 685

Gly Ala Ala Phe Ser Ala Gly Leu Thr Gly Gly Ile Ser Thr Ser Ile
690 695 700

Ala Val Phe Cys His Glu Leu Pro His Glu Leu Gly Asp Phe Ala Val
705 710 715 720

Leu Leu Lys Ala Gly Met Thr Val Lys Gln Ala Ile Val Tyr Asn Leu
725 730 735

Leu Ser Ala Met Met Ala Tyr Ile Gly Met Leu Ile Gly Thr Ala Val
740 745 750

Gly Gln Tyr Ala Asn Asn Ile Thr Leu Trp Ile Phe Ala Val Thr Ala
755 760 765

Gly Met Phe Leu Tyr Val Ala Leu Val Asp Met Leu Pro Glu Met Leu
770 775 780

His Gly Asp Gly Asp Asn Glu Glu His Gly Phe Cys Pro Val Gly Gln
785 790 795 800

Phe Ile Leu Gln Asn Leu Gly Leu Leu Phe Gly Phe Ala Ile Met Leu
805 810 815

Val Ile Ala Leu Tyr Glu Asp Lys Ile Val Phe Asp Ile Gln Phe
820 825 830

<210> 87
<211> 480
<212> PRT
<213> Homo sapiens

<400> 87
Met Leu Phe Arg Asn Arg Phe Leu Leu Leu Leu Ala Leu Ala Ala Leu
1 5 10 15

Leu Ala Phe Val Ser Leu Ser Leu Gln Phe Phe His Leu Ile Pro Val
20 25 30

Ser Thr Pro Lys Asn Gly Met Ser Ser Lys Ser Arg Lys Arg Ile Met
35 40 45

Pro Asp Pro Val Thr Glu Pro Pro Val Thr Asp Pro Val Tyr Glu Ala
50 55 60

Leu Leu Tyr Cys Asn Ile Pro Ser Val Ala Glu Arg Ser Met Glu Gly
65 70 75 80

His Ala Pro His His Phe Lys Leu Val Ser Val His Val Phe Ile Arg
85 90 95

His Gly Asp Arg Tyr Pro Leu Tyr Val Ile Pro Lys Thr Lys Arg Pro
100 105 110

```
Glu Ile Asp Cys Thr Leu Val Ala Asn Arg Lys Pro Tyr His Pro Lys
        115                 120                 125

Leu Glu Ala Phe Ile Ser His Met Ser Lys Gly Ser Gly Ala Ser Phe
    130                 135                 140

Glu Ser Pro Leu Asn Ser Leu Pro Leu Tyr Pro Asn His Pro Leu Cys
145                 150                 155                 160

Glu Met Gly Glu Leu Thr Gln Thr Gly Val Val Gln His Leu Gln Asn
                165                 170                 175

Gly Gln Leu Leu Arg Asp Ile Tyr Leu Lys Lys His Lys Leu Leu Pro
            180                 185                 190

Asn Asp Trp Ser Ala Asp Gln Leu Tyr Leu Glu Thr Thr Gly Lys Ser
        195                 200                 205

Arg Thr Leu Gln Ser Gly Leu Ala Leu Leu Tyr Gly Phe Leu Pro Asp
    210                 215                 220

Phe Asp Trp Lys Lys Ile Tyr Phe Arg His Gln Pro Ser Ala Leu Phe
225                 230                 235                 240

Cys Ser Gly Ser Cys Tyr Cys Pro Val Arg Asn Gln Tyr Leu Glu Lys
                245                 250                 255

Glu Gln Arg Arg Gln Tyr Leu Leu Arg Leu Lys Asn Ser Gln Leu Glu
            260                 265                 270

Lys Thr Tyr Gly Glu Met Ala Lys Ile Val Asp Val Pro Thr Lys Gln
        275                 280                 285

Leu Arg Ala Ala Asn Pro Ile Asp Ser Met Leu Cys His Phe Cys His
    290                 295                 300

Asn Val Ser Phe Pro Cys Thr Arg Asn Gly Cys Val Asp Met Glu His
305                 310                 315                 320

Phe Lys Val Ile Lys Thr His Gln Ile Glu Asp Glu Arg Glu Arg Arg
                325                 330                 335

Glu Lys Lys Leu Tyr Phe Gly Tyr Ser Leu Leu Gly Ala His Pro Ile
            340                 345                 350

Leu Asn Gln Thr Ile Gly Arg Met Gln Arg Ala Thr Glu Gly Arg Lys
        355                 360                 365

Glu Glu Leu Phe Ala Leu Tyr Ser Ala His Asp Val Thr Leu Ser Pro
    370                 375                 380

Val Leu Ser Ala Leu Gly Leu Ser Glu Ala Arg Phe Pro Arg Phe Ala
385                 390                 395                 400

Ala Arg Leu Ile Phe Glu Leu Trp Gln Asp Arg Glu Lys Pro Ser Glu
                405                 410                 415

His Ser Val Arg Ile Leu Tyr Asn Gly Val Asp Val Thr Phe His Thr
            420                 425                 430

Ser Phe Cys Gln Asp His His Lys Arg Ser Pro Lys Pro Met Cys Pro
        435                 440                 445

Leu Glu Asn Leu Val Arg Phe Val Lys Arg Asp Met Phe Val Ala Leu
    450                 455                 460
```

Gly Gly Ser Gly Thr Asn Tyr Tyr Asp Ala Cys His Arg Glu Gly Phe
465 470 475 480

<210> 88
<211> 151
<212> PRT
<213> Homo sapiens

<400> 88
Met Phe Leu Met Leu Gly Cys Ala Leu Pro Ile Tyr Asn Lys Tyr Trp
1 5 10 15

Pro Leu Phe Val Leu Phe Phe Tyr Ile Leu Ser Pro Ile Pro Tyr Cys
20 25 30

Ile Ala Arg Arg Leu Val Asp Asp Thr Asp Ala Met Ser Asn Ala Cys
35 40 45

Lys Glu Leu Ala Ile Phe Leu Thr Thr Gly Ile Val Val Ser Ala Phe
50 55 60

Gly Leu Pro Ile Val Phe Ala Arg Ala His Leu Met Gly Arg Leu Pro
65 70 75 80

Phe Phe Ser Lys Met Gly Thr Ala Glu Ser Glu Gly Arg Glu Thr Leu
85 90 95

Thr Gln Gln Leu Pro Leu Pro Ala Ala Ala Met Arg Arg Leu Leu Pro
100 105 110

Ala Ser Arg Val Ser Thr Gln Pro Val Leu Arg Leu Ala Asp Ser Ala
115 120 125

Glu Ser Leu Leu Gly Arg Pro Ala Leu Trp Ala Leu Gly Phe Leu Leu
130 135 140

Cys Pro Pro Ser Gln Ala Gln
145 150

<210> 89
<211> 132
<212> PRT
<213> Homo sapiens

<400> 89
Met Glu Ile Tyr Leu Ser Leu Gly Val Leu Ala Leu Gly Thr Leu Ser
1 5 10 15

Leu Leu Ala Val Thr Ser Leu Pro Ser Ile Ala Asn Ser Leu Asn Trp
20 25 30

Arg Glu Phe Ser Phe Val Gln Ser Ser Leu Gly Phe Val Ala Leu Val
35 40 45

Leu Ser Thr Leu His Thr Leu Thr Tyr Gly Trp Thr Arg Ala Phe Glu
50 55 60

Glu Ser Arg Tyr Lys Phe Tyr Leu Pro Pro Thr Phe Thr Leu Thr Leu
65 70 75 80

Leu Val Pro Cys Val Val Ile Leu Ala Lys Ala Leu Phe Leu Leu Pro

85 90 95

Cys Ile Ser Arg Arg Leu Ala Arg Ile Arg Arg Gly Trp Glu Arg Glu
100 105 110

Ser Thr Ile Lys Phe Thr Leu Pro Thr Asp His Ala Leu Ala Glu Lys
115 120 125

Thr Ser His Val
130

<210> 90
<211> 110
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (98)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (100)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 90
Met Ala Ala Pro Ala Leu Gly Leu Val Cys Gly Arg Cys Pro Glu Leu
1 5 10 15

Gly Leu Val Leu Leu Leu Leu Leu Leu Ser Leu Leu Cys Gly Ala Ala
20 25 30

Gly Ser Gln Glu Ala Gly Thr Gly Ala Gly Ala Gly Ser Leu Ala Gly
35 40 45

Ser Cys Gly Cys Gly Thr Pro Gln Arg Pro Gly Ala His Gly Ser Ser
50 55 60

Ala Ala Ala His Arg Tyr Ser Arg Glu Ala Asn Ala Pro Gly Pro Val
65 70 75 80

Pro Gly Glu Arg Gln Leu Ala His Ser Lys Val Leu His Arg Phe Leu
85 90 95

Arg Xaa Gly Xaa Gly Leu Leu Gly Ser Trp Thr Gly Leu Glu
100 105 110

<210> 91
<211> 188
<212> PRT
<213> Homo sapiens

<400> 91
Met Val Pro Gly Ala Ala Gly Trp Cys Cys Leu Val Leu Trp Leu Pro
1 5 10 15

Ala Cys Val Ala Ala His Gly Phe Arg Ile His Asp Tyr Leu Tyr Phe
20 25 30

Gln Val Leu Ser Pro Gly Asp Ile Arg Tyr Ile Phe Thr Ala Thr Pro
35 40 45

Ala Lys Asp Phe Gly Gly Ile Phe His Thr Arg Tyr Glu Gln Ile His

```
     50                  55                  60

Leu Val Pro Ala Glu Pro Pro Glu Ala Cys Gly Glu Leu Ser Asn Gly
 65                  70                  75                  80

Phe Phe Ile Gln Asp Gln Ile Ala Leu Val Glu Arg Gly Gly Cys Ser
                 85                  90                  95

Phe Leu Ser Lys Thr Arg Val Val Gln Glu His Gly Gly Arg Ala Val
            100                 105                 110

Ile Ile Ser Asp Asn Ala Val Asp Asn Asp Ser Phe Tyr Val Glu Met
        115                 120                 125

Ile Gln Asp Ser Thr Gln Arg Thr Ala Asp Ile Pro Ala Leu Phe Leu
    130                 135                 140

Leu Gly Arg Asp Gly Tyr Met Ile Arg Arg Ser Leu Glu Gln His Gly
145                 150                 155                 160

Leu Pro Trp Ala Ile Ile Ser Ile Pro Val Asn Val Thr Ser Ile Pro
                165                 170                 175

Thr Phe Glu Leu Leu Gln Pro Pro Trp Thr Phe Trp
            180                 185


<210> 92
<211> 179
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (143)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 92
Met Ala Gln Val Leu Ala Ser Glu Leu Ser Leu Val Ala Phe Ile Leu
  1               5                  10                  15

Leu Leu Val Met Ala Phe Ser Lys Lys Trp Leu Asp Leu Ser Arg Ser
             20                  25                  30

Leu Phe Tyr Gln Arg Trp Pro Val Asp Val Ser Asn Arg Ile His Thr
         35                  40                  45

Ser Ala His Val Met Ser Met Gly Leu Leu His Phe Cys Lys Ser Arg
     50                  55                  60

Ser Cys Ser Asp Leu Glu Asn Gly Lys Val Thr Phe Ile Phe Ser Thr
 65                  70                  75                  80

Leu Met Leu Phe Pro Ile Asn Ile Trp Ile Phe Glu Leu Glu Arg Asn
                 85                  90                  95

Val Ser Ile Pro Ile Gly Trp Ser Tyr Phe Ile Gly Trp Leu Val Leu
            100                 105                 110

Ile Leu Tyr Phe Thr Cys Ala Ile Leu Cys Tyr Phe Asn His Lys Ser
        115                 120                 125

Phe Trp Ser Leu Ile Leu Ser His Pro Ser Gly Ala Val Ser Xaa Ser
    130                 135                 140

Ser Ser Phe Gly Ser Val Glu Glu Ser Pro Arg Ala Gln Thr Ile Thr
```

145 150 155 160

Asp Thr Pro Ile Thr Gln Glu Gly Val Leu Asp Pro Glu Gln Lys Asp
165 170 175

Thr His Val

<210> 93
<211> 259
<212> PRT
<213> Homo sapiens

<400> 93
Met Val Ser Cys Ser Ile Leu Ala Leu Thr His Leu Leu Phe Glu Phe
1 5 10 15

Lys Gly Leu Met Gly Thr Ser Thr Val Glu Gln Leu Leu Glu Asn Val
20 25 30

Cys Leu Leu Leu Ala Ser Arg Thr Arg Asp Val Val Lys Ser Ala Leu
35 40 45

Gly Phe Ile Lys Val Ala Val Thr Val Met Asp Val Ala His Leu Ala
50 55 60

Lys His Val Gln Leu Val Met Glu Ala Ile Gly Lys Leu Ser Asp Asp
65 70 75 80

Met Arg Arg His Phe Arg Met Lys Leu Arg Asn Leu Phe Thr Lys Phe
85 90 95

Ile Arg Lys Phe Gly Phe Glu Leu Val Lys Arg Leu Leu Pro Glu Glu
100 105 110

Tyr His Arg Val Leu Val Asn Ile Arg Lys Ala Glu Ala Arg Ala Lys
115 120 125

Arg His Arg Ala Leu Ser Gln Ala Ala Val Glu Glu Glu Glu Glu Glu
130 135 140

Glu Glu Glu Glu Glu Pro Ala Gln Gly Lys Gly Asp Ser Ile Glu Glu
145 150 155 160

Ile Leu Ala Asp Ser Glu Asp Glu Glu Asp Asn Glu Glu Glu Glu Arg
165 170 175

Ser Arg Gly Lys Glu Gln Arg Lys Leu Ala Arg Gln Arg Ser Arg Ala
180 185 190

Trp Leu Lys Glu Gly Gly Gly Asp Glu Pro Leu Asn Phe Leu Asp Pro
195 200 205

Lys Val Ala Gln Arg Val Leu Ala Thr Gln Pro Gly Pro Ala Gly Gln
210 215 220

Glu Glu Gly Pro Gln Leu Gln Gly Glu Arg Arg Trp Pro Ala Asp His
225 230 235 240

Lys Gly Gly Gly Arg Arg Gln Gln Asp Gly Gly Arg Gly Arg Cys Gln
245 250 255

Arg Arg Arg

<210> 94
<211> 239
<212> PRT
<213> Homo sapiens

<400> 94
```
Met Ala Pro Leu Leu Pro Ser Leu Pro Leu His Leu His Thr Ser Leu
1               5                   10                  15

Cys Leu Arg Leu Cys Leu Ser Leu Ser Leu Ser Ala Trp Leu Ser Trp
            20                  25                  30

Ser Leu Pro Leu Cys Val Ser Leu Ser Ala Ser Tyr Pro Ala Trp Arg
        35                  40                  45

Leu Leu Pro Gln Leu His Gly Arg Ser Arg Glu Gln Arg Tyr Thr Lys
    50                  55                  60

Leu Ala Asp Trp Gln Tyr Ile Glu Glu Cys Val Gln Ala Ala Ser Pro
65                  70                  75                  80

Met Pro Leu Phe Gly Asn Gly Asp Ile Leu Ser Phe Glu Asp Ala Asn
                85                  90                  95

Arg Ala Met Gln Thr Gly Val Thr Gly Ile Met Ile Ala Arg Gly Ala
            100                 105                 110

Leu Leu Lys Pro Trp Leu Phe Thr Glu Ile Lys Glu Gln Arg His Trp
        115                 120                 125

Asp Ile Ser Ser Ser Glu Arg Leu Asp Ile Leu Arg Asp Phe Thr Asn
    130                 135                 140

Tyr Gly Leu Glu His Trp Gly Ser Asp Thr Gln Gly Val Glu Lys Thr
145                 150                 155                 160

Arg Arg Phe Leu Leu Glu Trp Leu Ser Phe Leu Cys Arg Tyr Val Pro
                165                 170                 175

Val Gly Leu Leu Glu Arg Leu Pro Gln Arg Ile Asn Glu Arg Pro Pro
            180                 185                 190

Tyr Tyr Leu Gly Arg Asp Tyr Leu Glu Thr Leu Met Ala Ser Gln Lys
        195                 200                 205

Ala Ala Asp Trp Ile Arg Ile Ser Glu Met Leu Leu Gly Pro Val Pro
    210                 215                 220

Pro Ser Phe Ala Phe Leu Pro Lys His Lys Ala Asn Ala Tyr Lys
225                 230                 235
```

<210> 95
<211> 138
<212> PRT
<213> Homo sapiens

<400> 95
```
Met Lys Met Met Val Val Leu Leu Met Leu Ser Ser Leu Ser Arg Leu
1               5                   10                  15

Leu Gly Leu Met Arg Pro Ser Ser Leu Arg Gln Tyr Leu Asp Ser Val
            20                  25                  30

Pro Leu Pro Pro Cys Gln Glu Gln Gln Pro Lys Ala Ser Ala Glu Leu
```

```
        35                  40                  45

Asp His Lys Ala Cys Tyr Leu Cys His Ser Leu Leu Met Leu Ala Gly
    50                  55                  60

Val Val Val Ser Cys Gln Asp Ile Thr Pro Asp Gln Trp Gly Glu Leu
 65                 70                  75                  80

Gln Leu Leu Cys Met Gln Leu Asp Arg His Ile Ser Thr Gln Ile Arg
                85                  90                  95

Glu Ser Pro Gln Ala Met His Arg Thr Met Leu Lys Asp Leu Ala Thr
            100                 105                 110

Gln Thr Tyr Ile Arg Trp Gln Glu Leu Leu Thr His Cys Gln Pro Gln
        115                 120                 125

Ala Gln Tyr Phe Ser Pro Trp Lys Asp Ile
    130                 135


<210> 96
<211> 122
<212> PRT
<213> Homo sapiens

<400> 96
Met Pro Pro Leu Ala Pro Gln Leu Cys Arg Ala Val Phe Leu Val Pro
  1               5                  10                  15

Ile Leu Leu Leu Leu Gln Val Lys Pro Leu Asn Gly Ser Pro Gly Pro
            20                  25                  30

Lys Asp Gly Ser Gln Thr Glu Lys Thr Pro Ser Ala Asp Gln Asn Gln
        35                  40                  45

Glu Gln Phe Glu Glu His Phe Val Ala Ser Ser Val Gly Glu Met Trp
    50                  55                  60

Gln Val Val Asp Met Ala Gln Gln Glu Glu Asp Gln Ser Ser Lys Thr
 65                 70                  75                  80

Ala Ala Val His Lys His Ser Phe His Leu Ser Phe Cys Phe Ser Leu
                85                  90                  95

Ala Ser Val Met Val Phe Ser Gly Gly Pro Leu Arg Arg Thr Phe Pro
            100                 105                 110

Asn Ile Gln Leu Cys Phe Met Leu Thr His
        115                 120


<210> 97
<211> 459
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (321)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (345)
<223> Xaa equals any of the naturally occurring L-amino acids
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```
<400> 97
Met Gly Gly Pro Arg Ala Trp Ala Leu Leu Cys Leu Gly Leu Leu Leu
 1               5                  10                  15

Pro Gly Gly Gly Ala Ala Trp Ser Ile Gly Ala Ala Pro Phe Ser Gly
            20                  25                  30

Arg Arg Asn Trp Cys Ser Tyr Val Val Thr Arg Thr Ile Ser Cys His
        35                  40                  45

Val Gln Asn Gly Thr Tyr Leu Gln Arg Val Leu Gln Asn Cys Pro Trp
    50                  55                  60

Pro Met Ser Cys Pro Gly Ser Ser Tyr Arg Thr Val Val Arg Pro Thr
65                  70                  75                  80

Tyr Lys Val Met Tyr Lys Ile Val Thr Ala Arg Glu Trp Arg Cys Cys
                85                  90                  95

Pro Gly His Ser Gly Val Ser Cys Glu Glu Val Ala Ala Ser Ser Ala
            100                 105                 110

Ser Leu Glu Pro Met Trp Ser Gly Ser Thr Met Arg Arg Met Ala Leu
        115                 120                 125

Arg Pro Thr Ala Phe Ser Gly Cys Leu Asn Cys Ser Lys Val Ser Glu
    130                 135                 140

Leu Thr Glu Arg Leu Lys Val Leu Glu Ala Lys Met Thr Met Leu Thr
145                 150                 155                 160

Val Ile Glu Gln Pro Val Pro Pro Thr Pro Ala Thr Pro Glu Asp Pro
                165                 170                 175

Ala Pro Leu Trp Gly Pro Pro Pro Ala Gln Gly Ser Pro Gly Asp Gly
            180                 185                 190

Gly Leu Gln Asp Gln Val Gly Ala Trp Gly Leu Pro Gly Pro Thr Gly
        195                 200                 205

Pro Lys Gly Asp Ala Gly Ser Arg Gly Pro Met Gly Met Arg Gly Pro
    210                 215                 220

Pro Gly Pro Gln Gly Pro Pro Gly Ser Pro Gly Arg Ala Gly Ala Val
225                 230                 235                 240

Gly Thr Pro Gly Glu Arg Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly
                245                 250                 255

Pro Pro Gly Pro Pro Ala Pro Val Gly Pro Pro His Ala Arg Ile Ser
            260                 265                 270

Gln His Gly Asp Pro Leu Leu Ser Asn Thr Phe Thr Glu Thr Asn Asn
        275                 280                 285

His Trp Pro Gln Gly Pro Thr Gly Pro Pro Gly Pro Pro Gly Pro Met
    290                 295                 300

Gly Pro Pro Gly Pro Pro Gly Pro Thr Gly Val Pro Gly Ser Pro Gly
305                 310                 315                 320

Xaa Ile Gly Pro Pro Gly Pro Thr Gly Pro Lys Gly Ile Ser Gly His
                325                 330                 335

Pro Gly Glu Lys Gly Glu Lys Lys Xaa Leu Arg Gly Glu Pro Gly Pro
```

```
            340                     345                     350

Gln Gly Ser Ala Gly Gln Arg Gly Glu Pro Gly Pro Lys Gly Asp Pro
        355                     360                     365

Gly Glu Lys Ser His Trp Asn Gln Ser Trp Gly Leu Gly Gly Pro Cys
    370                     375                     380

Arg His Arg His Pro Gln Pro Pro Ser Gly Gln Glu Gly Gly His Ala
385                     390                     395                     400

Thr Asn Tyr Arg Asp Arg Gly Pro Gln Glu Pro Gly Arg Glu Arg Leu
                405                     410                     415

Arg Val Val Ala Ala Pro Glu Ala Asp Gln Ala Arg Leu Pro Leu Leu
            420                     425                     430

Pro Gly Leu Gly Gln Leu Pro Pro Gly Thr Ala Arg Pro Tyr Leu Leu
        435                     440                     445

Met Ser Ser Gly Ser Leu Leu Pro Ser Arg Pro
    450                     455


<210> 98
<211> 352
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (284)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 98
Met Asp Phe Ile Gln His Leu Gly Val Cys Cys Leu Val Ala Leu Ile
  1               5                  10                  15

Ser Val Gly Leu Leu Ser Val Ala Ala Cys Trp Phe Leu Pro Ser Ile
             20                  25                  30

Ile Ala Ala Ala Ala Ser Trp Ile Ile Thr Cys Val Leu Leu Cys Cys
        35                  40                  45

Ser Lys His Ala Arg Cys Phe Ile Leu Leu Val Phe Leu Ser Cys Gly
    50                  55                  60

Leu Arg Glu Gly Arg Asn Ala Leu Ile Ala Ala Gly Thr Gly Ile Val
 65                  70                  75                  80

Ile Leu Gly His Val Glu Asn Ile Phe His Asn Phe Lys Gly Leu Leu
                 85                  90                  95

Asp Gly Met Thr Cys Asn Leu Arg Ala Lys Ser Phe Ser Ile His Phe
            100                 105                 110

Pro Leu Leu Lys Lys Tyr Ile Glu Ala Ile Gln Trp Ile Tyr Gly Leu
        115                 120                 125

Ala Thr Pro Leu Ser Val Phe Asp Asp Leu Val Ser Trp Asn Gln Thr
    130                 135                 140

Leu Ala Val Ser Leu Phe Ser Pro Ser His Val Leu Glu Ala Gln Leu
145                 150                 155                 160

Asn Asp Ser Lys Gly Glu Val Leu Ser Val Leu Tyr Gln Met Ala Thr
```

```
                165                 170                 175

Thr Thr Glu Val Leu Ser Ser Leu Gly Gln Lys Leu Leu Ala Phe Ala
            180                 185                 190

Gly Leu Ser Leu Val Leu Leu Gly Thr Gly Leu Phe Met Lys Arg Phe
        195                 200                 205

Leu Gly Pro Cys Gly Trp Lys Tyr Glu Asn Ile Tyr Ile Thr Arg Gln
    210                 215                 220

Phe Val Gln Phe Asp Glu Arg Glu Arg His Gln Gln Arg Pro Cys Val
225                 230                 235                 240

Leu Pro Leu Asn Lys Glu Glu Arg Arg Lys Tyr Val Ile Ile Pro Thr
                245                 250                 255

Phe Trp Pro Thr Pro Lys Glu Arg Lys Asn Leu Gly Leu Phe Phe Leu
            260                 265                 270

Pro Ile Leu Ile His Leu Cys Ile Trp Val Leu Xaa Ala Ala Val Asp
        275                 280                 285

Tyr Leu Leu Tyr Arg Leu Ile Phe Ser Val Ser Lys Gln Phe Gln Ser
    290                 295                 300

Leu Pro Gly Phe Glu Val His Leu Lys Leu His Gly Glu Lys Gln Gly
305                 310                 315                 320

Thr Gln Asp Ile Ile His Asp Ser Ser Phe Asn Ile Ser Val Phe Glu
                325                 330                 335

Pro Asn Cys Ile Pro Lys Pro Trp Gln Ala Leu Lys Leu Leu Ala His
            340                 345                 350
```

<210> 99
<211> 257
<212> PRT
<213> Homo sapiens

<400> 99

```
Met Glu Met Ile Ile Gln Phe Gly Phe Val Thr Leu Phe Val Ala Ser
  1               5                  10                  15

Phe Pro Leu Ala Pro Leu Phe Ala Leu Leu Asn Asn Ile Ile Glu Ile
            20                  25                  30

Arg Leu Asp Ala Lys Lys Phe Val Thr Glu Leu Arg Arg Pro Val Ala
        35                  40                  45

Val Arg Ala Lys Asp Ile Gly Ile Trp Tyr Asn Ile Leu Arg Gly Ile
    50                  55                  60

Gly Lys Leu Ala Val Ile Ile Asn Ala Phe Val Ile Ser Phe Thr Ser
 65                  70                  75                  80

Asp Phe Ile Pro Arg Leu Val Tyr Leu Tyr Met Tyr Ser Lys Asn Gly
                 85                  90                  95

Thr Met His Gly Phe Val Asn His Thr Leu Ser Ser Phe Asn Val Ser
            100                 105                 110
```

```
Asp Phe Gln Asn Gly Thr Ala Pro Asn Asp Pro Leu Asp Leu Gly Tyr
        115                 120                 125

Glu Val Gln Ile Cys Arg Tyr Lys Asp Tyr Arg Glu Pro Pro Trp Ser
    130                 135                 140

Glu Asn Lys Tyr Asp Ile Ser Lys Asp Phe Trp Ala Val Leu Ala Ala
145                 150                 155                 160

Arg Leu Ala Phe Val Ile Val Phe Gln Asn Leu Val Met Phe Met Ser
                165                 170                 175

Asp Phe Val Asp Trp Val Ile Pro Asp Ile Pro Lys Asp Ile Ser Gln
            180                 185                 190

Gln Ile His Lys Glu Lys Val Leu Met Val Glu Leu Phe Met Arg Glu
        195                 200                 205

Glu Gln Asp Lys Gln Gln Leu Leu Glu Thr Trp Met Glu Lys Glu Arg
    210                 215                 220

Gln Lys Asp Glu Pro Pro Cys Asn His His Asn Thr Lys Ala Cys Pro
225                 230                 235                 240

Asp Ser Leu Gly Ser Pro Ala Pro Ser His Ala Tyr His Gly Gly Val
                245                 250                 255

Leu


<210> 100
<211> 127
<212> PRT
<213> Homo sapiens

<400> 100
Met Ala Gln Tyr Ile Leu Val Ile Ile Leu Ile Ser Phe Cys Ser Asp
  1               5                  10                  15

Ser Leu Ser Gly Arg Ala Gln Asn Gly Thr Glu Ile Asn Gln Thr Val
            20                  25                  30

Ile Leu Ile Cys Ser Leu Arg Phe Phe Lys Ser Glu Ala Ile Asp Ala
        35                  40                  45

Cys Leu Met His Pro His Thr Ala Cys Leu Thr Gly Asp Ala Thr Leu
    50                  55                  60

Leu Ser Ser Ser Ala Met Lys His Lys Arg Gln Arg Lys Ser Arg Tyr
 65                  70                  75                  80

Thr Ser His Arg Glu His Phe Arg Val Pro Gln Arg Trp Trp Gln Glu
                85                  90                  95

Ala His Ser Arg Val Ser Ile Arg Val Cys Val Trp Val Ser Gly Ile
            100                 105                 110

Ser Val Ala Pro Ile Phe Leu His Cys Ser Glu His Pro Val Leu
        115                 120                 125


<210> 101
<211> 136
<212> PRT
<213> Homo sapiens
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```
<400> 101
Met Leu Met Leu Leu Thr Leu Leu Val Leu Gly Met Val Trp Val Ala
 1               5                  10                  15

Ser Ala Ile Val Asp Lys Asn Lys Ala Asn Arg Glu Ser Leu Tyr Asp
            20                  25                  30

Phe Trp Glu Tyr Tyr Leu Pro Tyr Leu Tyr Ser Cys Ile Ser Phe Leu
        35                  40                  45

Gly Val Leu Leu Leu Leu Gly Glu Cys Thr Gly Ser Gly Arg Glu Trp
    50                  55                  60

Ala Gly Ser Leu Asp Gln Ser Asn Gln Ala Arg Arg Lys Gly Asn Gly
65                  70                  75                  80

Gly His Val Arg Glu Gly Val Glu Ser Arg Val Trp Gln Val Thr Gly
                85                  90                  95

Ser Cys Pro Tyr Ser Val Tyr Ser Thr Gly Ser Arg Pro His Val Leu
            100                 105                 110

Arg His Trp Glu Ala Ala Ser Gln Ala Pro Ala Ala Gly Arg Pro Gly
        115                 120                 125

Gly Ala Ala Val Leu Leu Ser Leu
    130                 135


<210> 102
<211> 144
<212> PRT
<213> Homo sapiens

<400> 102
Met Ser Ala Val Ser Ala Pro Ala Leu Trp Gln Thr Trp Cys Val Pro
 1               5                  10                  15

Ala Ala Arg Ala Trp Thr Ser Ser Thr Leu Arg His Asp Ala Val Ala
            20                  25                  30

Arg Pro Asn Pro Ser Thr Ser Leu Thr Pro Gly Leu Leu Thr Ser Ser
        35                  40                  45

Asp Ser Pro Arg Trp Pro Gly Leu Gln Glu Ala Pro Gly Arg Pro Cys
    50                  55                  60

Ile Arg Leu Gly Arg Ser Glu Leu Cys Met Tyr Ile Tyr Thr Tyr Ile
65                  70                  75                  80

Asp Thr Phe Ile Ile Tyr Thr His Ser Leu Tyr Ile Tyr Ile His Cys
                85                  90                  95

Phe Leu Ala Pro Glu Leu Ile Trp Val Gln Ala His Phe Lys Thr Leu
            100                 105                 110

Pro Gly Gly Gly Cys Phe Phe Ser Gly Phe Leu Ala Arg Glu Glu Gly
        115                 120                 125

Glu Gly Thr Gly Trp Val Phe Ser Leu Lys Arg Glu Ser Arg Arg Phe
    130                 135                 140
```

<210> 103
<211> 151
<212> PRT
<213> Homo sapiens

<400> 103
Met Leu His Trp Val Leu Ser Phe Phe Phe Leu Leu Ser Cys Pro Arg
1 5 10 15

Thr Glu Gly Leu Pro Gly Leu Tyr Cys Pro Gly Cys Ser Gln Cys Pro
20 25 30

Gly Arg Gly Met Trp Pro Gly Asp Pro Gly Pro Gly Ile Gln Gly Pro
35 40 45

Gly Leu Asp Leu Arg Thr Gly Met Glu Ala Thr Gly Ala Gln Gln Pro
50 55 60

Thr Leu Ser Ser Pro His Cys Leu Leu Ser Leu Pro Thr Leu Pro Ala
65 70 75 80

Arg Ala Val Gln Leu Arg Trp Asp Leu Ser Ile Ser Arg Ala Gly Gly
85 90 95

Arg Val Ala Val Leu Gly Leu Cys Leu Glu Pro Gly Gly Ser Leu Leu
100 105 110

Leu Pro Pro Ser Ala Leu Pro Glu Thr Asp Pro Cys Ala Ala Cys Pro
115 120 125

Pro Cys Pro Phe Val Pro Met Ser Gly Gly Gly Gly Arg Pro Thr Val
130 135 140

Pro Glu Ala Gly His Gln Pro
145 150

<210> 104
<211> 112
<212> PRT
<213> Homo sapiens

<400> 104
Met Ala Tyr Leu Thr Leu Phe Gln Met Gly Ser Trp Met Ser Phe Ser
1 5 10 15

Leu Ser Leu Cys Ser Leu Leu Phe Ile Leu Thr Gly His Cys Leu Ser
20 25 30

Glu Asn Phe Tyr Val Arg Gly Asp Gly Thr Arg Ala Tyr Phe Phe Thr
35 40 45

Lys Gly Glu Val His Ser Met Phe Cys Lys Ala Ser Leu Asp Glu Lys
50 55 60

Gln Asn Leu Val Asp Arg Arg Leu Gln Val Asn Arg Lys Lys Gln Val
65 70 75 80

Lys Met His Arg Val Trp Ile Gln Gly Lys Phe Gln Lys Pro Leu His
85 90 95

Gln Thr Gln Asn Ser Ser Asn Met Val Ser Thr Leu Leu Ser Gln Asp
100 105 110

<210> 105
<211> 80
<212> PRT
<213> Homo sapiens

<400> 105
Met Trp Pro Arg Met Leu Ala Phe Ser Thr Trp Leu Glu Trp Leu Leu
1 5 10 15

Phe Ser Pro Leu Pro Gln Ser Val Gly Cys Pro Gly Pro Leu Glu Phe
20 25 30

Tyr Cys Val Gln Asp Arg Arg Pro Pro Ser Leu Pro Asp Gly Ala Asp
35 40 45

His Phe Ser Ser Pro Thr Arg Ile Thr Ser Ser Ser Ile Ser Pro Ala
50 55 60

Leu Ser Leu Gln Ala Pro Glu Ala Gly Gly Phe Leu Ser Ile Pro Gly
65 70 75 80

<210> 106
<211> 51
<212> PRT
<213> Homo sapiens

<400> 106
Met Ser Leu Glu Pro Ser Thr Ser Ser Phe Asn Ile Leu Leu Phe Pro
1 5 10 15

Ala Phe Leu Arg Val Phe Gly Trp Ala Leu Gly Trp Met Pro Trp Glu
20 25 30

Tyr Leu Tyr Leu Ser Ser Lys Val Thr Asn Gly Glu Thr Gly Thr Gln
35 40 45

Arg Gly Thr
50

<210> 107
<211> 60
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (10)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (48)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 107
Met Phe Phe Pro Cys Leu Pro Thr Leu Xaa Leu Arg Ile Leu His Ser
1 5 10 15

Gly Trp Val Gly Leu Phe Leu Leu Ile Ser Ser Arg Ala Pro Ser Ser
20 25 30

Ser Leu Ala Trp Lys His Gly Pro Gly Glu Leu Trp Trp Pro Arg Xaa
35 40 45

Pro Leu Arg Ser Cys Thr Gly Leu Ala Ser Cys Gly
50 55 60

<210> 108
<211> 54
<212> PRT
<213> Homo sapiens

<400> 108
Met Trp Pro Phe Leu His Leu Leu Asn Met Pro Phe Thr Leu Thr Gln
1 5 10 15

Val Val Ala Ser Pro Ser Ser Cys Ser Asn Trp Lys Pro Gln His Pro
20 25 30

Glu Met Pro Pro Pro Gln Ile His Cys Thr His Val Cys Leu Cys Met
35 40 45

Arg Val Cys Ala Arg Val
50

<210> 109
<211> 97
<212> PRT
<213> Homo sapiens

<400> 109
Met Leu Trp Lys Leu Lys Leu Ser Arg Cys Trp Leu Asp Leu Thr Leu
1 5 10 15

Leu Ile Phe Ser Gln Ile Ser His Met Asp Gln Ile Ile Phe Phe Phe
20 25 30

Val Val Tyr Pro Ile Leu Asn Asn Ile Phe Ser Leu Asn Tyr Cys Arg
35 40 45

Asp Phe Phe Cys Gly Gly Tyr Phe Leu Phe Cys Ser Lys Ile Ile Arg
50 55 60

Cys Lys Ala Ile Leu Cys Leu Thr Val Ala Leu Ser Lys Gln Leu Cys
65 70 75 80

Ser Gly Val Ala Phe Asp Val Leu Glu Phe Asp Tyr Met Gln Ser Cys
85 90 95

Ile

<210> 110
<211> 122
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (63)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (99)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (122)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 110
Met Met Thr Met Thr Ser Asp Arg Trp Phe Ser Met Ala Trp Ala Ser
1 5 10 15

Cys Ser Leu Ser Arg Pro Pro Leu Thr Pro Ser Cys Ser Cys Gln Gln
20 25 30

Pro Ala Thr Val Ala Leu Leu Leu Gln Thr Ile Ser Val Cys Ser Ala
35 40 45

Gln Gln Ala Asp Pro Leu Ser Pro Pro Arg Ala Cys Arg Pro Xaa Arg
50 55 60

Gln Phe Pro Val Leu Gln Ser Ala Gly Pro Pro His Ser Pro His Val
65 70 75 80

Tyr Ala Phe Val Leu Phe Pro Val Ser Ser Arg Trp Gln Gly Gly Asp
85 90 95

Phe Cys Xaa Ile Cys Cys Cys Phe Pro Gln Cys Leu Gly Arg Cys Leu
100 105 110

Glu His Thr Arg Cys Ser Ile Asn Pro Xaa
115 120

<210> 111
<211> 53
<212> PRT
<213> Homo sapiens

<400> 111
Met Ser Thr Phe Val Cys Val Cys Val Phe Cys Phe Val Leu Arg Ser
1 5 10 15

Glu Ala Arg Ala Lys Arg Lys Gln Asp Gln Arg Asn Thr Lys Arg Cys
20 25 30

Leu Leu Thr Lys Gly Gln Arg Asp Leu Ser Val Asn Gln Ser Lys Ile
35 40 45

Asn Arg Thr Ala Asn
50

<210> 112
<211> 80
<212> PRT
<213> Homo sapiens

<400> 112
Met Gly Trp Ile Asp Leu Leu Leu Pro Glu Leu Gly Ala Leu Arg Val
1 5 10 15

Phe Leu His Leu Phe Leu Val Ala Leu Arg Thr Lys Arg Trp Ile Phe

20 25 30

Arg Thr Leu Gly Gln Leu Thr Cys Val Asn Ile Leu Gly Asp Ser Arg
35 40 45

Lys Lys Arg Glu Cys Arg Leu Asn Lys Arg Gln Leu Gln Phe Gly Glu
50 55 60

Lys Thr Leu Gln Val Pro Glu Arg Leu Val Val Arg His Ser Pro Phe
65 70 75 80

<210> 113
<211> 72
<212> PRT
<213> Homo sapiens

<400> 113
Met Leu Val Leu Phe Lys Phe Leu Pro Leu Thr Ser Ser Gly Arg Phe
1 5 10 15

Leu Ser Val Thr Leu Tyr His Arg Val His His Gln Thr Phe Phe Ala
20 25 30

Gly Ala Lys Ser Phe Ser Pro Ala Ser Thr Leu Asn Leu Tyr Ile Cys
35 40 45

Ser Ser Gln Phe Gln Ser Leu Gln Lys Leu Tyr Cys Gly Val Ile Pro
50 55 60

Val Leu Arg Tyr Ala Ser Ile Glu
65 70

<210> 114
<211> 45
<212> PRT
<213> Homo sapiens

<400> 114
Met Val Thr Ser Gly Met Leu Val Phe Ser Ile Lys Thr Phe Ser Ser
1 5 10 15

Lys Ala Phe Leu Ala Val Val Ser Phe Ile Leu Val Val Ser Ile Lys
20 25 30

Cys Ser Glu Gly Ala Asp Thr Ser Arg Lys Gly Phe Ser
35 40 45

<210> 115
<211> 74
<212> PRT
<213> Homo sapiens

<400> 115
Met Val Leu Leu Leu Leu Leu Leu Leu Gln Lys Ile Pro Gly Thr Pro
1 5 10 15

Leu Phe Gln Pro Gly Phe Leu Gly Trp Ala Gln Glu Ser Cys Gln Ile
20 25 30

Gln Ser Tyr Val Gly Ser Lys Leu Pro Leu Cys Cys Phe Cys Gln Ala

35 40 45

Arg Cys Gly His Ser Lys Phe Ile Cys Val Asn Lys Arg Lys Glu Glu
50 55 60

Pro Ser Gly Cys Asn Arg Thr Asp Ser Ser
65 70

<210> 116
<211> 41
<212> PRT
<213> Homo sapiens

<400> 116
Met Asn Leu Met Val Arg Leu Leu Ala Leu Gly Leu Ile Ser Gly Met
1 5 10 15

Met Ser Asn Ile Thr Gln Ser His Ser Ser Lys Ile Ser Ala Phe Gly
20 25 30

Ile Phe Ile Gly Pro Glu Gln Phe Leu
35 40

<210> 117
<211> 82
<212> PRT
<213> Homo sapiens

<400> 117
Met Asn Arg Ser Thr Arg Ser Tyr Arg Cys Trp Ala Thr Trp Pro Arg
1 5 10 15

Leu Gly Trp Ala Leu Pro Cys Cys Met Asn Ser Leu Arg Lys Gly Arg
20 25 30

Lys Phe Ser Gln Ile Thr Thr Ser Leu Met Ala Ser Val Ser Ser Ala
35 40 45

Ser Met Val Ser Arg Arg Arg Arg Pro Leu Pro Lys His Pro Val Thr
50 55 60

Thr Thr Ser Thr Ala Thr Ala Leu Leu Gly Thr Ser Ser Thr Trp Ser
65 70 75 80

Lys Ser

<210> 118
<211> 53
<212> PRT
<213> Homo sapiens

<400> 118
Met Gly Gln Arg Gly Val Phe Leu Leu Ile Leu Asp Ala Phe Ser Val
1 5 10 15

Pro Ser Thr Ala Ser Cys Leu Ile Thr Pro Leu Pro Pro Pro His Pro
20 25 30

Gln Pro Ser Gln Phe Phe Leu Ala Ser Ala Leu Gln Pro Tyr Leu Gly
35 40 45

Lys Glu Glu Trp Val

50

<210> 119
<211> 180
<212> PRT
<213> Homo sapiens

<400> 119
Met Ala Ile Cys Ser Cys Gln Cys Pro Ala Ala Met Ala Phe Cys Phe
1 5 10 15

Leu Glu Thr Leu Trp Trp Glu Phe Thr Ala Ser Tyr Asp Thr Thr Cys
20 25 30

Ile Gly Leu Ala Ser Arg Pro Tyr Ala Phe Leu Glu Phe Asp Ser Ile
35 40 45

Ile Gln Lys Val Lys Trp His Phe Asn Tyr Val Ser Ser Ser Gln Met
50 55 60

Glu Cys Ser Leu Glu Lys Ile Gln Glu Glu Leu Lys Leu Gln Pro Pro
65 70 75 80

Ala Val Leu Thr Leu Glu Asp Thr Asp Val Ala Asn Gly Val Met Asn
85 90 95

Gly His Thr Pro Met His Leu Glu Pro Ala Pro Asn Phe Arg Met Glu
100 105 110

Pro Val Thr Ala Leu Gly Ile Leu Ser Leu Ile Leu Asn Ile Met Cys
115 120 125

Ala Ala Leu Asn Leu Ile Arg Gly Val His Leu Ala Glu His Ser Leu
130 135 140

Gln Val Ala His Glu Glu Ile Gly Asn Ile Leu Ala Phe Leu Val Pro
145 150 155 160

Phe Val Ala Cys Ile Phe Gln Asp Pro Arg Ser Trp Phe Cys Trp Leu
165 170 175

Asp Gln Thr Ser
180

<210> 120
<211> 599
<212> PRT
<213> Homo sapiens

<400> 120
Met Glu Leu Leu Gly Pro Val Pro Pro Glu Gln Gln Phe Ile Asn Gln
1 5 10 15

Lys Met Arg Pro Gly Ser Gly Met Leu Ser Ile Arg Val Ile Pro Asp
20 25 30

Gly Pro Thr Arg Ala Leu Gln Ile Thr Asp Phe Cys His Arg Lys Ser
35 40 45

Ser Arg Ser Tyr Glu Val Asp Glu Leu Pro Val Thr Glu Gln Glu Leu
50 55 60

Gln Lys Leu Lys Asn Pro Asp Thr Glu Gln Glu Leu Glu Val Leu Val
65 70 75 80

Arg Leu Glu Gly Gly Ile Gly Leu Ser Leu Ile Asn Lys Val Pro Glu
85 90 95

Glu Leu Val Phe Ala Ser Leu Thr Gly Ile Asn Val His Tyr Thr Gln
100 105 110

Leu Ala Thr Ser His Met Leu Glu Leu Ser Ile Gln Asp Val Gln Val
115 120 125

Asp Asn Gln Leu Ile Gly Thr Thr Gln Pro Phe Met Leu Tyr Val Thr
130 135 140

Pro Leu Ser Asn Glu Asn Glu Val Ile Glu Thr Gly Pro Ala Val Gln
145 150 155 160

Val Asn Ala Val Lys Phe Pro Ser Lys Ser Ala Leu Thr Asn Ile Tyr
165 170 175

Lys His Leu Met Ile Thr Ala Gln Arg Phe Thr Val Gln Ile Glu Glu
180 185 190

Lys Leu Leu Leu Lys Leu Leu Ser Phe Phe Gly Tyr Asp Gln Ala Glu
195 200 205

Ser Glu Val Glu Lys Tyr Asp Glu Asn Leu His Glu Lys Thr Ala Glu
210 215 220

Gln Gly Gly Thr Pro Ile Arg Tyr Tyr Phe Glu Asn Leu Lys Ile Ser
225 230 235 240

Ile Pro Gln Ile Lys Leu Ser Val Phe Thr Ser Asn Lys Leu Pro Leu
245 250 255

Asp Leu Lys Ala Leu Lys Ser Thr Leu Gly Phe Pro Leu Ile Arg Phe
260 265 270

Glu Asp Ala Val Ile Asn Leu Asp Pro Phe Thr Arg Val His Pro Tyr
275 280 285

Glu Thr Lys Glu Phe Ile Ile Asn Asp Ile Leu Lys His Phe Gln Glu
290 295 300

Glu Leu Leu Ser Gln Ala Ala Arg Ile Leu Gly Ser Val Asp Phe Leu
305 310 315 320

Gly Asn Pro Met Gly Leu Leu Asn Asp Val Ser Glu Gly Val Thr Gly
325 330 335

Leu Ile Lys Tyr Gly Asn Val Gly Gly Leu Ile Arg Asn Val Thr His
340 345 350

Gly Val Ser Asn Ser Ala Gly Lys Phe Ala Gly Thr Leu Ser Asp Gly
355 360 365

Leu Gly Lys Thr Met Asp Asn Arg His Gln Ser Glu Arg Glu Tyr Ile
370 375 380

Arg Tyr His Ala Ala Thr Ser Gly Glu His Leu Val Ala Gly Ile His
385 390 395 400

Gly Leu Ala His Gly Ile Ile Gly Gly Leu Thr Ser Val Ile Thr Ser
405 410 415

Thr Val Glu Gly Val Lys Thr Glu Gly Gly Val Ser Gly Phe Ile Ser
420 425 430

```
Gly Leu Gly Lys Gly Leu Val Gly Thr Val Thr Lys Pro Val Ala Gly
        435                 440                 445

Ala Leu Asp Phe Ala Ser Glu Thr Ala Gln Ala Val Arg Asp Thr Ala
    450                 455                 460

Thr Leu Ser Gly Pro Arg Thr Gln Ala Gln Arg Val Arg Lys Pro Arg
465                 470                 475                 480

Cys Cys Thr Gly Pro Gln Gly Leu Leu Pro Arg Tyr Ser Glu Ser Gln
                485                 490                 495

Ala Glu Gly Gln Glu Gln Leu Phe Lys Leu Thr Asp Asn Ile Gln Asp
            500                 505                 510

Glu Phe Phe Ile Ala Val Glu Asn Ile Asp Ser Tyr Cys Val Leu Ile
        515                 520                 525

Ser Ser Lys Ala Val Tyr Phe Leu Lys Ser Gly Asp Tyr Val Asp Arg
    530                 535                 540

Glu Ala Ile Phe Leu Glu Val Lys Tyr Asp Asp Leu Leu Pro Leu Pro
545                 550                 555                 560

Cys Leu Gln Arg Pro Trp Glu Gly Val Cys Ala Gly Asp Gln Glu Ser
                565                 570                 575

Arg Glu His Glu Gln Trp Ser Val His Pro Arg Pro Leu Pro Pro Glu
            580                 585                 590

Ala His Gly Pro Cys Glu Ile
        595


<210> 121
<211> 45
<212> PRT
<213> Homo sapiens

<400> 121
Met Tyr Pro Pro Val Ala Pro Ser Phe Trp Gly Cys Val Cys Phe Phe
  1               5                  10                  15

Trp Ala Val Pro Leu Val Cys Cys Arg Asp Ser Trp Lys Gly Leu Ser
            20                  25                  30

Leu Phe Val Gly Ser Gly Gly Leu Gly Leu Val Glu His
        35                  40                  45


<210> 122
<211> 57
<212> PRT
<213> Homo sapiens

<400> 122
Met Phe Phe Gln Gly Trp Val Asp Arg Trp Leu Leu Gly Cys Leu Ala
  1               5                  10                  15

Pro Gly Gly Phe Ala Ile His Glu Ala Arg Ala Gly Asn Thr Val Ser
            20                  25                  30

Leu Pro Met Val Asp Pro Cys Glu Cys Gln Glu Ala Ser Ser Ser Val
        35                  40                  45
```

Leu Glu Met Ile Ser Ala Thr Ile Leu
50 55

<210> 123
<211> 50
<212> PRT
<213> Homo sapiens

<400> 123
Met Thr His Gly Cys Leu Ser Leu Ala Ser Met Ala Ala Gly Leu Gly
1 5 10 15

Ser Val Ser Leu Phe Leu Phe Val Gln Gln Trp Thr Pro Thr Thr Ala
20 25 30

Ser Thr Gly Glu Thr Pro Ser Ser Trp Gln Lys Thr Thr Ser Cys Val
35 40 45

Arg Arg
50

<210> 124
<211> 74
<212> PRT
<213> Homo sapiens

<400> 124
Met His Trp Thr Phe Ser Ser Ser Leu Gly Cys Leu Tyr His Phe Ser
1 5 10 15

Leu Ser Phe Ser Gly Leu His Thr Val Leu Lys Ser Ser Pro Ser Ser
20 25 30

Arg Phe Leu Leu Pro Cys Ser Ser Gln Val Thr Gln Pro Ser Pro Val
35 40 45

Gly Gln Pro Arg Leu Val Val Gln Leu Pro Pro Val Lys Val Ile Gly
50 55 60

His Arg Thr Gly Gln Cys Arg Gly Pro Gly
65 70

<210> 125
<211> 253
<212> PRT
<213> Homo sapiens

<400> 125
Met Asp Asn Arg Phe Ala Thr Ala Phe Val Ile Ala Cys Val Leu Ser
1 5 10 15

Leu Ile Ser Thr Ile Tyr Met Ala Ala Ser Ile Gly Thr Asp Phe Trp
20 25 30

Tyr Glu Tyr Arg Ser Pro Val Gln Glu Asn Ser Ser Asp Leu Asn Lys
35 40 45

Ser Ile Trp Asp Glu Phe Ile Ser Asp Glu Ala Asp Glu Lys Thr Tyr
50 55 60

Asn Asp Ala Leu Phe Arg Tyr Asn Gly Thr Val Gly Leu Trp Arg Arg
65 70 75 80

```
Cys Ile Thr Ile Pro Lys Asn Met His Trp Tyr Ser Pro Pro Glu Arg
                85                  90                  95

Thr Glu Ser Phe Asp Val Val Thr Lys Cys Val Ser Phe Thr Leu Thr
            100                 105                 110

Glu Gln Phe Met Glu Lys Phe Val Asp Pro Gly Asn His Asn Ser Gly
        115                 120                 125

Ile Asp Leu Leu Arg Thr Tyr Leu Trp Arg Cys Gln Phe Leu Leu Pro
    130                 135                 140

Phe Val Ser Leu Gly Leu Met Cys Phe Gly Ala Leu Ile Gly Leu Cys
145                 150                 155                 160

Ala Cys Ile Cys Arg Ser Leu Tyr Pro Thr Ile Ala Thr Gly Ile Leu
                165                 170                 175

His Leu Leu Ala Gly Leu Cys Thr Leu Gly Ser Val Ser Cys Tyr Val
            180                 185                 190

Ala Gly Ile Glu Leu Leu His Gln Lys Leu Glu Leu Pro Asp Asn Val
        195                 200                 205

Ser Gly Glu Phe Gly Trp Ser Phe Cys Leu Ala Cys Val Ser Ala Pro
    210                 215                 220

Leu Gln Phe Met Ala Ser Ala Leu Phe Ile Trp Ala Ala His Thr Asn
225                 230                 235                 240

Arg Lys Glu Tyr Thr Leu Met Lys Ala Tyr Arg Val Ala
                245                 250
```

<210> 126
<211> 89
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (87)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 126

```
Met Phe Leu Leu Arg Pro Leu Pro Ile Leu Leu Val Thr Gly Gly Gly
  1               5                  10                  15

Tyr Ala Gly Tyr Arg Gln Tyr Glu Lys Tyr Arg Glu Arg Glu Leu Glu
             20                  25                  30

Lys Leu Gly Leu Glu Ile Pro Pro Lys Leu Ala Gly His Trp Glu Val
         35                  40                  45

Ala Leu Tyr Lys Ser Val Pro Thr Arg Leu Leu Ser Arg Ala Trp Gly
    50                  55                  60

Arg Leu Asn Gln Val Glu Leu Pro His Trp Leu Arg Arg Pro Val Tyr
 65                  70                  75                  80

Ser Leu Tyr Ile Trp Thr Xaa Gly Gly
                85
```

<210> 127
<211> 49

<212> PRT
<213> Homo sapiens

<400> 127
Met Gln Val Ser Ser Trp Val Val Phe Gln Leu Val Trp Asn Ser Leu
1 5 10 15

Val Leu Thr Gln Thr Gly Ile Lys His Tyr Phe Arg Phe Ser Leu Cys
20 25 30

Gln Phe Leu Ser Ser Tyr Asn His Val Asn Gln Asp Val Arg Thr Ser
35 40 45

Ile

<210> 128
<211> 90
<212> PRT
<213> Homo sapiens

<400> 128
Met Asp Trp Ala Val Leu Thr Val Val Leu Gly Pro Cys Val Pro Gly
1 5 10 15

Leu Ser Gly Ser Pro Pro Trp Pro Leu Pro Ser Ser His Leu Leu Glu
20 25 30

Ala Lys Leu Cys Glu Thr Trp His Ser Phe Gln Thr Ser Val Pro Pro
35 40 45

Arg Pro Cys Ala Gly Val Thr Pro Glu Leu Arg Met Ser Ala Arg Ser
50 55 60

Arg Gln Tyr Arg Glu Gly Thr Gln Arg Lys Ala Ser Gln Leu Ser Lys
65 70 75 80

Asp Arg Asp Arg Leu Trp Ser Gly Arg Ala
85 90

<210> 129
<211> 94
<212> PRT
<213> Homo sapiens

<400> 129
Met Trp Pro Trp Trp Leu Met Val Glu Arg Thr Val Val Leu Leu Leu
1 5 10 15

Ile Thr Tyr Leu Val Pro Val Gly Gly Ser Ala Val Gly Pro Pro Gly
20 25 30

Pro Gly Cys Asn Val Ser Thr Ser Pro Pro Pro Pro Ala Thr Arg Cys
35 40 45

Pro Asp Glu Ser Glu Leu Tyr Arg Asp Pro Gly Glu Ala Pro Leu Glu
50 55 60

Ala Asp Gln Ala Glu Arg Gly Ala Ala His Glu Gly Gly His Pro Gly
65 70 75 80

Arg Asp Pro Trp Gly Ala Arg Arg Gly Pro Pro Arg Cys Gly
85 90

<210> 130
<211> 78
<212> PRT
<213> Homo sapiens

<400> 130
Met Ser Pro His Gln Pro Met Gln Val Ser Ser Ser Lys Thr Ile Leu
1 5 10 15

Trp Leu Val Leu Ser Cys Leu Cys Pro Ser Ser Pro His Pro Val Ile
20 25 30

Ser Gly Leu Pro Gln Trp Tyr Ile Gly Val Leu Ala Gly Ile Val Pro
35 40 45

Val Ala Pro Ile Arg Pro Gly Asp Ser Gly Leu Asp Leu Gln Arg Glu
50 55 60

Gly Pro Gln Pro Ile Leu Ser Gln Gly Leu Asn Arg Arg Thr
65 70 75

<210> 131
<211> 615
<212> PRT
<213> Homo sapiens

<400> 131
Met Ile Leu Phe Leu Leu Ala Phe Leu Leu Phe Cys Gly Leu Leu Phe
1 5 10 15

Tyr Ile Asn Leu Ala Asp His Trp Lys Ala Leu Ala Phe Arg Leu Glu
20 25 30

Glu Glu Gln Lys Met Arg Pro Glu Ile Ala Gly Leu Lys Pro Ala Asn
35 40 45

Pro Pro Val Leu Pro Ala Pro Gln Lys Ala Asp Thr Asp Pro Glu Asn
50 55 60

Leu Pro Glu Ile Ser Ser Gln Lys Thr Gln Arg His Ile Gln Arg Gly
65 70 75 80

Pro Pro His Leu Gln Ile Arg Pro Pro Ser Gln Asp Leu Lys Asp Gly
85 90 95

Thr Gln Glu Glu Ala Thr Lys Arg Gln Glu Ala Pro Val Asp Pro Arg
100 105 110

Pro Glu Gly Asp Pro Gln Arg Thr Val Ile Ser Trp Arg Gly Ala Val
115 120 125

Ile Glu Pro Glu Gln Gly Thr Glu Leu Pro Ser Arg Arg Ala Glu Val
130 135 140

Pro Thr Lys Pro Pro Leu Pro Pro Ala Arg Thr Gln Gly Thr Pro Val
145 150 155 160

His Leu Asn Tyr Arg Gln Lys Gly Val Ile Asp Val Phe Leu His Ala
165 170 175

Trp Lys Gly Tyr Arg Lys Phe Ala Trp Gly His Asp Glu Leu Lys Pro
180 185 190

Val Ser Arg Ser Phe Ser Glu Trp Phe Gly Leu Gly Leu Thr Leu Ile

```
        195                 200                 205

Asp Ala Leu Asp Thr Met Trp Ile Leu Gly Leu Arg Lys Glu Phe Glu
    210                 215                 220

Glu Ala Arg Lys Trp Val Ser Lys Lys Leu His Phe Glu Lys Asp Val
225                 230                 235                 240

Asp Val Asn Leu Phe Glu Ser Thr Ile Arg Ile Leu Gly Gly Leu Leu
                245                 250                 255

Ser Ala Tyr His Leu Ser Gly Asp Ser Leu Phe Leu Arg Lys Ala Glu
            260                 265                 270

Asp Phe Gly Asn Arg Leu Met Pro Ala Phe Arg Thr Pro Ser Lys Ile
        275                 280                 285

Pro Tyr Ser Asp Val Asn Ile Gly Thr Gly Val Ala His Pro Pro Arg
    290                 295                 300

Trp Thr Ser Asp Ser Thr Val Ala Glu Val Thr Ser Ile Gln Leu Glu
305                 310                 315                 320

Phe Arg Glu Leu Ser Arg Leu Thr Gly Asp Lys Lys Phe Gln Glu Ala
                325                 330                 335

Val Glu Lys Val Thr Gln His Ile His Gly Leu Ser Gly Lys Lys Asp
            340                 345                 350

Gly Leu Val Pro Met Phe Ile Asn Thr His Ser Gly Leu Phe Thr His
        355                 360                 365

Leu Gly Val Phe Thr Leu Gly Ala Arg Ala Asp Ser Tyr Tyr Glu Tyr
    370                 375                 380

Leu Leu Lys Gln Trp Ile Gln Gly Gly Lys Gln Glu Thr Gln Leu Leu
385                 390                 395                 400

Glu Asp Tyr Val Glu Ala Ile Glu Gly Val Arg Thr His Leu Leu Arg
                405                 410                 415

His Ser Glu Pro Ser Lys Leu Thr Phe Val Gly Glu Leu Ala His Gly
            420                 425                 430

Arg Phe Ser Ala Lys Met Asp His Leu Val Cys Phe Leu Pro Gly Thr
        435                 440                 445

Leu Ala Leu Gly Val Tyr His Gly Leu Pro Ala Ser His Met Glu Leu
    450                 455                 460

Ala Gln Glu Leu Met Glu Thr Cys Tyr Gln Met Asn Arg Gln Met Glu
465                 470                 475                 480

Thr Gly Leu Ser Pro Glu Ile Val His Phe Asn Leu Tyr Pro Gln Pro
                485                 490                 495

Gly Arg Arg Asp Val Glu Val Lys Pro Ala Asp Arg His Asn Leu Leu
            500                 505                 510

Arg Pro Glu Thr Val Glu Ser Leu Phe Tyr Leu Tyr Arg Val Thr Gly
        515                 520                 525

Asp Arg Lys Tyr Gln Asp Trp Gly Trp Glu Ile Leu Gln Ser Phe Ser
    530                 535                 540

Arg Phe Thr Arg Val Pro Ser Gly Gly Tyr Ser Ser Ile Asn Asn Val
```

545 550 555 560

Gln Asp Pro Gln Lys Pro Glu Pro Arg Asp Lys Met Glu Ser Phe Phe
565 570 575

Leu Gly Glu Thr Leu Lys Tyr Leu Phe Leu Leu Phe Ser Asp Asp Pro
580 585 590

Asn Leu Leu Ser Leu Asp Ala Tyr Val Phe Asn Thr Glu Ala His Pro
595 600 605

Leu Pro Ile Trp Thr Pro Ala
610 615

<210> 132
<211> 42
<212> PRT
<213> Homo sapiens

<400> 132
Met Leu Trp Leu Gly Thr Ser Leu Ile Phe Ser Ser Phe Ser Ala Ser
1 5 10 15

Phe Asp Gly Val Pro Phe Leu Ser Ser Trp Leu Phe Trp Ser Ser Gly
20 25 30

Ser Ser Pro Asn Ser Leu Ile Pro Pro Phe
35 40

<210> 133
<211> 99
<212> PRT
<213> Homo sapiens

<400> 133
Met Glu Gly Pro Arg Gly Trp Leu Val Leu Cys Val Leu Ala Ile Ser
1 5 10 15

Leu Ala Ser Met Val Thr Glu Asp Leu Cys Arg Ala Pro Asp Gly Lys
20 25 30

Lys Gly Glu Ala Gly Arg Pro Gly Arg Arg Gly Arg Pro Gly Leu Lys
35 40 45

Gly Glu Gln Gly Glu Pro Gly Ala Pro Gly Ile Arg Thr Gly Ile Gln
50 55 60

Gly Leu Lys Gly Asp Gln Gly Glu Pro Gly Pro Ser Gly Asn Pro Gly
65 70 75 80

Lys Val Gly Tyr Pro Gly Pro Ser Gly Pro Leu Arg Ser Pro Trp His
85 90 95

Pro Gly Asn

<210> 134
<211> 57
<212> PRT
<213> Homo sapiens

<400> 134
Met Gly His Leu His Trp Gly Val Ser Gly Asn Phe Phe Phe Pro Arg

```
1               5                   10                  15

Leu Ser Leu Phe Leu Leu Phe Ala Trp Leu Gln Ile Thr Gln Ala Asn
            20                  25                  30

Glu Pro Arg Leu Pro Gly Lys Tyr Ser Ile Lys Ala Ile Lys Ile Thr
        35                  40                  45

Ile Cys Ile Thr Phe Arg Thr Ser Ala
    50                  55


&lt;210&gt; 135
&lt;211&gt; 316
&lt;212&gt; PRT
&lt;213&gt; Homo sapiens

&lt;400&gt; 135
Met Leu Arg Arg Arg Gly Ser Pro Gly Met Gly Val His Val Gly Ala
1               5                   10                  15

Ala Leu Gly Ala Leu Trp Phe Cys Leu Thr Gly Ala Leu Glu Val Gln
            20                  25                  30

Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr Leu
        35                  40                  45

Cys Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn
    50                  55                  60

Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala
65                  70                  75                  80

Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe
                85                  90                  95

Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val
            100                 105                 110

Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp
        115                 120                 125

Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys
    130                 135                 140

Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr
145                 150                 155                 160

Val Thr Ile Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val
                165                 170                 175

Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr
            180                 185                 190

Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Ile Leu
        195                 200                 205

Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn
    210                 215                 220

Pro Val Leu Gln Gln Asp Ala His Ser Ser Val Thr Ile Thr Gly Gln
225                 230                 235                 240

Pro Met Thr Phe Pro Pro Glu Ala Leu Trp Val Thr Val Gly Leu Ser
                245                 250                 255
```

```
Val Cys Leu Ile Ala Leu Leu Val Ala Leu Ala Phe Val Cys Trp Arg
            260                 265                 270

Lys Ile Lys Gln Ser Cys Glu Glu Glu Asn Ala Gly Ala Glu Asp Gln
        275                 280                 285

Asp Gly Glu Gly Glu Gly Ser Lys Thr Ala Leu Gln Pro Leu Lys His
    290                 295                 300

Ser Asp Ser Lys Glu Asp Asp Gly Gln Glu Ile Ala
305                 310                 315


<210> 136
<211> 302
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (128)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 136
Met Arg Leu Gly Ser Pro Gly Leu Leu Phe Leu Leu Phe Ser Ser Leu
  1               5                  10                  15

Arg Ala Asp Thr Gln Glu Lys Glu Val Arg Ala Met Val Gly Ser Asp
            20                  25                  30

Val Glu Leu Ser Cys Ala Cys Pro Glu Gly Ser Arg Phe Asp Leu Asn
        35                  40                  45

Asp Val Tyr Val Tyr Trp Gln Thr Ser Glu Ser Lys Thr Val Val Thr
    50                  55                  60

Tyr His Ile Pro Gln Asn Ser Ser Leu Glu Asn Val Asp Ser Arg Tyr
 65                  70                  75                  80

Arg Asn Arg Ala Leu Met Ser Pro Ala Gly Met Leu Arg Gly Asp Phe
                85                  90                  95

Ser Leu Arg Leu Phe Asn Val Thr Pro Gln Asp Glu Gln Lys Phe His
            100                 105                 110

Cys Leu Val Leu Ser Gln Ser Leu Gly Phe Gln Glu Val Leu Ser Xaa
        115                 120                 125

Glu Val Thr Leu His Val Ala Ala Asn Phe Ser Val Pro Val Val Ser
    130                 135                 140

Ala Pro His Ser Pro Ser Gln Asp Glu Leu Thr Phe Thr Cys Thr Ser
145                 150                 155                 160

Ile Asn Gly Tyr Pro Arg Pro Asn Val Tyr Trp Ile Asn Lys Thr Asp
                165                 170                 175

Asn Ser Leu Leu Asp Gln Ala Leu Gln Asn Asp Thr Val Phe Leu Asn
            180                 185                 190

Met Arg Gly Leu Tyr Asp Val Val Ser Val Leu Arg Ile Ala Arg Thr
        195                 200                 205

Pro Ser Val Asn Ile Gly Cys Cys Ile Glu Asn Val Leu Leu Gln Gln
    210                 215                 220
```

```
Asn Leu Thr Val Gly Ser Gln Thr Gly Asn Asp Ile Gly Glu Arg Asp
225                 230                 235                 240

Lys Ile Thr Glu Asn Pro Val Ser Thr Gly Glu Lys Asn Ala Ala Thr
                245                 250                 255

Trp Ser Ile Leu Ala Val Leu Cys Leu Leu Val Val Val Ala Val Ala
            260                 265                 270

Ile Gly Trp Val Cys Arg Asp Arg Cys Leu Gln His Ser Tyr Ala Gly
        275                 280                 285

Ala Trp Ala Val Ser Pro Glu Thr Glu Leu Thr Gly His Val
    290                 295                 300


<210> 137
<211> 374
<212> PRT
<213> Homo sapiens

<400> 137
Met Ala Ala Pro Ala Leu Gly Leu Val Cys Gly Arg Cys Pro Glu Leu
  1               5                  10                  15

Gly Leu Val Leu Leu Leu Leu Leu Leu Ser Leu Leu Cys Gly Ala Ala
             20                  25                  30

Gly Ser Gln Glu Ala Gly Thr Gly Ala Gly Ala Gly Ser Leu Ala Gly
         35                  40                  45

Ser Cys Gly Cys Gly Thr Pro Gln Arg Pro Gly Ala His Gly Ser Ser
     50                  55                  60

Ala Ala Ala His Arg Tyr Ser Arg Glu Ala Asn Ala Pro Gly Pro Val
 65                  70                  75                  80

Pro Gly Glu Arg Gln Leu Ala His Ser Lys Met Val Pro Ile Pro Ala
                 85                  90                  95

Gly Val Phe Thr Met Gly Thr Asp Asp Pro Gln Ile Lys Gln Asp Gly
            100                 105                 110

Glu Ala Pro Ala Arg Arg Val Thr Ile Asp Ala Phe Tyr Met Asp Ala
        115                 120                 125

Tyr Glu Val Ser Asn Thr Glu Phe Glu Lys Phe Val Asn Ser Thr Gly
    130                 135                 140

Tyr Leu Thr Glu Ala Glu Lys Phe Gly Asp Ser Phe Val Phe Glu Gly
145                 150                 155                 160

Met Leu Ser Glu Gln Val Lys Thr Asn Ile Gln Gln Ala Val Ala Ala
                165                 170                 175

Ala Pro Trp Trp Leu Pro Val Lys Gly Ala Asn Trp Arg His Pro Glu
            180                 185                 190

Gly Pro Asp Ser Thr Ile Leu His Arg Pro Asp His Pro Val Leu His
        195                 200                 205

Val Ser Trp Asn Asp Ala Val Ala Tyr Cys Thr Trp Ala Gly Lys Arg
    210                 215                 220

Leu Pro Thr Glu Ala Glu Trp Glu Tyr Ser Cys Arg Gly Gly Leu His
225                 230                 235                 240
```

```
Asn Arg Leu Phe Pro Trp Gly Asn Lys Leu Gln Pro Lys Gly Gln His
                245                 250                 255

Tyr Ala Asn Ile Trp Gln Gly Glu Phe Pro Val Thr Asn Thr Gly Glu
            260                 265                 270

Asp Gly Phe Gln Gly Thr Ala Pro Val Asp Ala Phe Pro Pro Asn Gly
        275                 280                 285

Tyr Gly Leu Tyr Asn Ile Val Gly Asn Ala Trp Glu Trp Thr Ser Asp
    290                 295                 300

Trp Trp Thr Val His His Ser Val Glu Glu Thr Leu Asn Pro Lys Gly
305                 310                 315                 320

Pro Pro Ser Gly Lys Asp Arg Val Lys Lys Gly Gly Ser Tyr Met Cys
                325                 330                 335

His Arg Ser Tyr Cys Tyr Arg Tyr Arg Cys Ala Ala Arg Ser Gln Asn
            340                 345                 350

Thr Pro Asp Ser Ser Ala Ser Asn Leu Gly Phe Arg Cys Ala Ala Asp
        355                 360                 365

Arg Leu Pro Thr Met Asp
    370


<210> 138
<211> 127
<212> PRT
<213> Homo sapiens

<400> 138
Met Val Pro Gly Ala Ala Gly Trp Cys Cys Leu Val Leu Trp Leu Pro
  1               5                  10                  15

Ala Cys Val Ala Ala His Gly Phe Arg Ile His Asp Tyr Leu Tyr Phe
             20                  25                  30

Gln Val Leu Ser Pro Gly Asp Ile Arg Tyr Ile Phe Thr Ala Thr Pro
         35                  40                  45

Ala Lys Asp Phe Gly Gly Ile Phe His Thr Arg Tyr Glu Gln Ile His
     50                  55                  60

Leu Val Pro Ala Glu Pro Pro Glu Ala Cys Gly Glu Leu Ser Asn Gly
 65                  70                  75                  80

Phe Phe Ile Gln Asp Gln Ile Ala Leu Val Glu Arg Gly Gly Cys Ser
                 85                  90                  95

Phe Leu Ser Lys Thr Arg Val Val Gln Glu His Gly Gly Arg Ala Val
            100                 105                 110

Ile Ile Ser Asp Asn Ala Leu Thr Met Thr Ala Ser Thr Trp Arg
        115                 120                 125


<210> 139
<211> 122
<212> PRT
<213> Homo sapiens

<400> 139
```

```
Met Pro Pro Leu Ala Pro Gln Leu Cys Arg Ala Val Phe Leu Val Pro
 1               5                  10                  15

Ile Leu Leu Leu Leu Gln Val Lys Pro Leu Asn Gly Ser Pro Gly Pro
            20                  25                  30

Lys Asp Gly Ser Gln Thr Glu Lys Thr Pro Ser Ala Asp Gln Asn Gln
        35                  40                  45

Glu Gln Phe Glu Glu His Phe Val Ala Ser Ser Val Gly Glu Met Trp
    50                  55                  60

Gln Val Val Asp Met Ala Gln Gln Glu Glu Asp Gln Ser Ser Lys Thr
65                  70                  75                  80

Ala Ala Val His Lys His Ser Phe His Leu Ser Phe Cys Phe Ser Leu
                85                  90                  95

Ala Ser Val Met Val Phe Ser Gly Gly Pro Leu Arg Arg Thr Phe Pro
            100                 105                 110

Asn Ile Gln Leu Cys Phe Met Leu Thr His
        115                 120


<210> 140
<211> 257
<212> PRT
<213> Homo sapiens

<400> 140
Met Asp Phe Ile Gln His Leu Gly Val Cys Cys Leu Val Ala Leu Ile
 1               5                  10                  15

Ser Val Gly Leu Leu Ser Val Ala Ala Cys Trp Phe Leu Pro Ser Ile
            20                  25                  30

Ile Ala Ala Ala Ala Ser Trp Ile Ile Thr Cys Val Leu Leu Cys Cys
        35                  40                  45

Ser Lys His Ala Arg Cys Phe Ile Leu Leu Val Phe Leu Ser Cys Gly
    50                  55                  60

Leu Arg Glu Gly Arg Asn Ala Leu Ile Ala Ala Gly Thr Gly Ile Val
65                  70                  75                  80

Ile Leu Gly His Val Glu Asn Ile Phe His Asn Phe Lys Gly Leu Leu
                85                  90                  95

Asp Gly Met Thr Cys Asn Leu Arg Ala Lys Ser Phe Ser Ile His Phe
            100                 105                 110

Pro Leu Leu Lys Lys Tyr Ile Glu Ala Ile Gln Trp Ile Tyr Gly Leu
        115                 120                 125

Ala Thr Pro Leu Ser Val Phe Asp Asp Leu Val Ser Trp Asn Gln Thr
    130                 135                 140

Leu Ala Val Ser Leu Phe Ser Pro Ser His Val Leu Glu Ala Gln Leu
145                 150                 155                 160

Asn Asp Ser Lys Gly Glu Val Leu Ser Val Leu Tyr Gln Met Ala Thr
                165                 170                 175

Thr Thr Glu Val Leu Ser Ser Leu Gly Gln Lys Leu Leu Ala Phe Ala
            180                 185                 190
```

```
Gly Leu Ser Leu Val Leu Leu Gly Thr Gly Leu Phe Met Lys Arg Phe
        195                 200                 205

Leu Gly Pro Cys Gly Trp Lys Tyr Glu Asn Ile Tyr Ile Thr Arg Gln
    210                 215                 220

Phe Val Gln Phe Asp Glu Arg Glu Arg His Gln Gln Arg Pro Cys Val
225                 230                 235                 240

Leu Pro Leu Asn Lys Glu Glu Arg Arg Lys Phe Ile Ser Gly Phe Gln
                245                 250                 255

Ser


<210> 141
<211> 257
<212> PRT
<213> Homo sapiens

<400> 141
Met Asp Phe Ile Gln His Leu Gly Val Cys Cys Leu Val Ala Leu Ile
  1               5                  10                  15

Ser Val Gly Leu Leu Ser Val Ala Ala Cys Trp Phe Leu Pro Ser Ile
            20                  25                  30

Ile Ala Ala Ala Ala Ser Trp Ile Ile Thr Cys Val Leu Leu Cys Cys
        35                  40                  45

Ser Lys His Ala Arg Cys Phe Ile Leu Leu Val Phe Leu Ser Cys Gly
    50                  55                  60

Leu Arg Glu Gly Arg Asn Ala Leu Ile Ala Ala Gly Thr Gly Ile Val
65                  70                  75                  80

Ile Leu Gly His Val Glu Asn Ile Phe His Asn Phe Lys Gly Leu Leu
                85                  90                  95

Asp Gly Met Thr Cys Asn Leu Arg Ala Lys Ser Phe Ser Ile His Phe
            100                 105                 110

Pro Leu Leu Lys Lys Tyr Ile Glu Ala Ile Gln Trp Ile Tyr Gly Leu
        115                 120                 125

Ala Thr Pro Leu Ser Val Phe Asp Asp Leu Val Ser Trp Asn Gln Thr
    130                 135                 140

Leu Ala Val Ser Leu Phe Ser Pro Ser His Val Leu Glu Ala Gln Leu
145                 150                 155                 160

Asn Asp Ser Lys Gly Glu Val Leu Ser Val Leu Tyr Gln Met Ala Thr
                165                 170                 175

Thr Thr Glu Val Leu Ser Ser Leu Gly Gln Lys Leu Leu Ala Phe Ala
            180                 185                 190

Gly Leu Ser Leu Val Leu Leu Gly Thr Gly Leu Phe Met Lys Arg Phe
        195                 200                 205

Leu Gly Pro Cys Gly Trp Lys Tyr Glu Asn Ile Tyr Ile Thr Arg Gln
    210                 215                 220

Phe Val Gln Phe Asp Glu Arg Glu Arg His Gln Gln Arg Pro Cys Val
```

225 230 235 240

Leu Pro Leu Asn Lys Glu Glu Arg Arg Lys Phe Ile Ser Gly Phe Gln
245 250 255

Ser

<210> 142
<211> 291
<212> PRT
<213> Homo sapiens

<400> 142
Met Asp Phe Ile Gln His Leu Gly Val Cys Cys Leu Val Ala Leu Ile
1 5 10 15

Ser Val Gly Leu Leu Ser Val Ala Ala Cys Trp Phe Leu Pro Ser Ile
20 25 30

Ile Ala Ala Ala Ala Ser Trp Ile Ile Thr Cys Val Leu Leu Cys Cys
35 40 45

Ser Lys His Ala Arg Cys Phe Ile Leu Leu Val Phe Leu Ser Cys Gly
50 55 60

Leu Arg Glu Gly Arg Asn Ala Leu Ile Ala Ala Gly Thr Gly Ile Val
65 70 75 80

Ile Leu Gly His Val Glu Asn Ile Phe His Asn Phe Lys Gly Leu Leu
85 90 95

Asp Gly Met Thr Cys Asn Leu Arg Ala Lys Ser Phe Ser Ile His Phe
100 105 110

Pro Leu Leu Lys Lys Tyr Ile Glu Ala Ile Gln Trp Ile Tyr Gly Leu
115 120 125

Ala Thr Pro Leu Ser Val Phe Asp Asp Leu Val Ser Trp Asn Gln Thr
130 135 140

Leu Ala Val Ser Leu Phe Ser Pro Ser His Val Leu Glu Ala Gln Leu
145 150 155 160

Asn Asp Ser Lys Gly Glu Val Leu Ser Val Leu Tyr Gln Met Ala Thr
165 170 175

Thr Thr Glu Val Leu Ser Ser Leu Gly Gln Lys Leu Leu Ala Phe Ala
180 185 190

Gly Leu Ser Leu Val Leu Leu Gly Thr Gly Leu Phe Met Lys Arg Phe
195 200 205

Leu Gly Pro Cys Gly Trp Lys Tyr Glu Asn Ile Tyr Ile Thr Arg Gln
210 215 220

Phe Val Gln Phe Asp Glu Arg Glu Arg His Gln Gln Arg Pro Cys Met
225 230 235 240

Leu Pro Leu Asn Lys Glu Glu Arg Arg Lys Asn Lys Glu Leu Lys Ile
245 250 255

Leu Ser Met Ile Leu Pro Leu Ile Tyr Leu Cys Leu Asn Pro Thr Val
260 265 270

Ser Gln Asn Gln Asn Ser Phe Tyr Leu Arg Pro Gly Phe Leu Ser Val
275 280 285

Leu Phe Phe
290

<210> 143
<211> 21
<212> PRT
<213> Homo sapiens

<400> 143
Met His Asp Val Leu Phe Phe Leu Ser Phe Ser Leu Val Ala Cys Val
1 5 10 15

Lys Ala Gly Met Leu
20

<210> 144
<211> 173
<212> PRT
<213> Homo sapiens

<400> 144
Met Pro Pro Tyr Thr Pro Phe Phe Gly Thr Arg Ala Leu Leu Ser Val
1 5 10 15

Ser Leu Pro Pro Pro Cys Met Leu His Trp Val Leu Ser Phe Phe Phe
20 25 30

Leu Leu Ser Cys Pro Arg Thr Glu Gly Leu Pro Gly Leu Tyr Cys Pro
35 40 45

Gly Cys Ser Gln Cys Pro Gly Arg Gly Met Trp Pro Gly Asp Pro Gly
50 55 60

Pro Gly Ile Gln Gly Pro Gly Leu Asp Leu Arg Thr Gly Met Glu Ala
65 70 75 80

Thr Gly Ala Gln Gln Pro Thr Leu Ser Ser Pro His Cys Leu Leu Ser
85 90 95

Leu Pro Thr Leu Pro Ala Arg Ala Val Gln Leu Arg Trp Asp Leu Ser
100 105 110

Ile Ser Arg Ala Gly Gly Arg Val Ala Val Leu Gly Leu Cys Leu Glu
115 120 125

Pro Gly Gly Ser Leu Leu Leu Pro Pro Ser Ala Leu Pro Glu Thr Asp
130 135 140

Pro Cys Ala Ala Cys Pro Pro Cys Pro Phe Val Pro Met Ser Gly Gly
145 150 155 160

Gly Gly Arg Pro Thr Val Pro Glu Ala Gly His Gln Pro
165 170

<210> 145
<211> 60
<212> PRT
<213> Homo sapiens

<220>

<221> SITE
<222> (10)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (42)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 145
Met Phe Phe Pro Cys Leu Pro Thr Leu Xaa Leu Arg Ile Leu His Ser
1 5 10 15

Gly Trp Val Gly Leu Phe Leu Leu Ile Ser Ser Arg Ala Pro Ser Ser
20 25 30

Ser Leu Ala Trp Lys His Gly Pro Gly Xaa Leu Trp Trp Pro Arg Arg
35 40 45

Pro Leu Arg Ser Cys Thr Gly Leu Ala Ser Cys Gly
50 55 60

<210> 146
<211> 45
<212> PRT
<213> Homo sapiens

<400> 146
Met Val Thr Ser Gly Met Leu Val Phe Ser Ile Lys Thr Phe Ser Ser
1 5 10 15

Lys Ala Phe Leu Ala Val Val Ser Phe Ile Leu Val Val Ser Ile Lys
20 25 30

Cys Ser Glu Gly Ala Asp Thr Ser Arg Lys Gly Phe Ser
35 40 45

<210> 147
<211> 404
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (41)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (77)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (96)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (98)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE

<222> (108)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (122)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (124)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (126)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (175)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (192)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (210)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (236)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (239)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (309)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (335)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (389)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 147
Met His Pro Ile Pro Ser Ser Phe Met Ile Lys Ala Val Ser Ser Phe
1 5 10 15

Leu Thr Ala Glu Glu Ala Ser Val Gly Asn Pro Glu Gly Ala Phe Met
20 25 30

Lys Val Leu Gln Ala Arg Lys Asn Xaa Thr Ser Thr Glu Leu Ile Val

```
        35                  40                  45

Glu Pro Glu Glu Pro Ser Asp Ser Ser Gly Ile Asn Leu Ser Gly Phe
    50                  55                  60

Gly Ser Glu Gln Leu Asp Thr Asn Asp Glu Ser Asp Xaa Ile Ser Thr
65                  70                  75                  80

Leu Ser Tyr Ile Leu Pro Tyr Phe Ser Ala Val Asn Leu Asp Val Xaa
                85                  90                  95

Ser Xaa Leu Leu Pro Phe Ile Lys Leu Pro Thr Xaa Gly Asn Ser Leu
            100                 105                 110

Ala Lys Ile Gln Thr Val Gly Gln Asn Xaa Gln Xaa Val Xaa Arg Val
        115                 120                 125

Leu Met Gly Pro Arg Ser Ile Gln Lys Arg His Phe Lys Glu Val Gly
    130                 135                 140

Arg Gln Ser Ile Arg Arg Glu Gln Gly Ala Gln Ala Ser Val Glu Asn
145                 150                 155                 160

Ala Ala Glu Glu Lys Arg Leu Gly Ser Pro Ala Pro Arg Glu Xaa Glu
                165                 170                 175

Gln Pro His Thr Gln Gln Gly Pro Glu Lys Leu Ala Gly Asn Ala Xaa
            180                 185                 190

Tyr Thr Lys Pro Ser Phe Thr Gln Glu His Lys Ala Ala Val Ser Val
        195                 200                 205

Leu Xaa Pro Phe Ser Lys Gly Ala Pro Ser Thr Ser Ser Pro Ala Lys
    210                 215                 220

Ala Leu Pro Gln Val Arg Asp Arg Trp Lys Asp Xaa Thr His Xaa Ile
225                 230                 235                 240

Ser Ile Leu Glu Ser Ala Lys Ala Arg Val Thr Asn Met Lys Ala Ser
                245                 250                 255

Lys Pro Ile Ser His Ser Arg Lys Lys Tyr Arg Phe His Lys Thr Arg
            260                 265                 270

Ser Arg Met Thr His Arg Thr Pro Lys Val Lys Lys Ser Pro Lys Phe
        275                 280                 285

Arg Lys Lys Ser Tyr Leu Ser Arg Leu Met Leu Ala Asn Arg Pro Pro
    290                 295                 300

Phe Ser Ala Ala Xaa Ser Leu Ile Asn Ser Pro Ser Gln Gly Ala Phe
305                 310                 315                 320

Ser Ser Leu Gly Asp Leu Ser Pro Gln Glu Asn Pro Phe Leu Xaa Val
                325                 330                 335

Ser Ala Pro Ser Glu His Phe Ile Glu Thr Thr Asn Ile Lys Asp Thr
            340                 345                 350

Thr Ala Arg Asn Ala Leu Glu Glu Asn Val Phe Met Glu Asn Thr Asn
        355                 360                 365

Met Pro Glu Val Thr Ile Ser Glu Asn Thr Asn Tyr Asn His Pro Pro
    370                 375                 380

Glu Ala Asp Ser Xaa Gly Thr Ala Phe Asn Leu Gly Pro Thr Val Lys
```

385 390 395 400

Gln Thr Glu Thr

<210> 148
<211> 53
<212> PRT
<213> Homo sapiens

<400> 148
Met Gly Gln Arg Gly Val Phe Leu Leu Ile Leu Asp Ala Phe Ser Val
1 5 10 15

Pro Ser Thr Ala Ser Cys Leu Ile Thr Pro Leu Pro Pro Pro His Pro
20 25 30

Gln Pro Ser Gln Phe Phe Leu Ala Ser Ala Leu Gln Pro Tyr Leu Gly
35 40 45

Lys Glu Glu Trp Val
50

<210> 149
<211> 53
<212> PRT
<213> Homo sapiens

<400> 149
Met Gly Gln Arg Gly Val Phe Leu Leu Ile Leu Asp Ala Phe Ser Val
1 5 10 15

Pro Ser Thr Ala Ser Cys Leu Ile Thr Pro Leu Pro Pro Pro His Pro
20 25 30

Gln Pro Ser Gln Phe Phe Leu Ala Ser Ala Leu Gln Pro Tyr Leu Gly
35 40 45

Lys Glu Glu Trp Val
50

<210> 150
<211> 50
<212> PRT
<213> Homo sapiens

<400> 150
Met Thr His Gly Cys Leu Ser Leu Ala Ser Met Ala Ala Gly Leu Gly
1 5 10 15

Ser Val Ser Leu Phe Leu Phe Val Gln Gln Trp Thr Pro Thr Thr Ala
20 25 30

Ser Thr Gly Glu Thr Pro Ser Ser Trp Gln Lys Thr Thr Ser Cys Val
35 40 45

Arg Arg
50

<210> 151
<211> 253
<212> PRT

<213> Homo sapiens

<400> 151
Met Asp Asn Arg Phe Ala Thr Ala Phe Val Ile Ala Cys Val Leu Ser
1   5   10   15

Leu Ile Ser Thr Ile Tyr Met Ala Ala Ser Ile Gly Thr Asp Phe Trp
            20   25   30

Tyr Glu Tyr Arg Ser Pro Val Gln Glu Asn Ser Ser Asp Leu Asn Lys
        35   40   45

Ser Ile Trp Asp Glu Phe Ile Ser Asp Glu Ala Asp Glu Lys Thr Tyr
    50   55   60

Asn Asp Ala Leu Phe Arg Tyr Asn Gly Thr Val Gly Leu Trp Arg Arg
65   70   75   80

Cys Ile Thr Ile Pro Lys Asn Met His Trp Tyr Ser Pro Pro Glu Arg
                85   90   95

Thr Glu Ser Phe Asp Val Val Thr Lys Cys Val Ser Phe Thr Leu Thr
            100   105   110

Glu Gln Phe Met Glu Lys Phe Val Asp Pro Gly Asn His Asn Ser Gly
        115   120   125

Ile Asp Leu Leu Arg Thr Tyr Leu Trp Arg Cys Gln Phe Leu Leu Pro
    130   135   140

Phe Val Ser Leu Gly Leu Met Cys Phe Gly Ala Leu Ile Gly Leu Cys
145   150   155   160

Ala Cys Ile Cys Arg Ser Leu Tyr Pro Thr Ile Ala Thr Gly Ile Leu
                165   170   175

His Leu Leu Ala Gly Leu Cys Thr Leu Gly Ser Val Ser Cys Tyr Val
            180   185   190

Ala Gly Ile Glu Leu Leu His Gln Lys Leu Glu Leu Pro Asp Asn Val
        195   200   205

Ser Gly Glu Phe Gly Trp Ser Phe Cys Leu Ala Cys Val Ser Ala Pro
    210   215   220

Leu Gln Phe Met Ala Ser Ala Leu Phe Ile Trp Ala Ala His Thr Asn
225   230   235   240

Arg Lys Glu Tyr Thr Leu Met Lys Ala Tyr Arg Val Ala
                245   250

<210> 152
<211> 127
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (107)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (109)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (116)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 152
Met Phe Leu Leu Arg Pro Leu Pro Ile Leu Leu Val Thr Gly Gly Gly
1   5   10   15

Tyr Ala Gly Tyr Arg Gln Tyr Glu Lys Tyr Arg Glu Arg Glu Leu Glu
            20   25   30

Lys Leu Gly Leu Glu Ile Pro Pro Lys Leu Ala Gly His Trp Glu Val
        35   40   45

Ala Leu Tyr Lys Ser Val Pro Thr Arg Leu Leu Ser Arg Ala Trp Gly
    50   55   60

Arg Leu Asn Gln Val Glu Leu Pro His Trp Leu Arg Arg Pro Val Tyr
65   70   75   80

Ser Leu Tyr Ile Trp Thr Phe Gly Val Asn Met Lys Glu Ala Ala Val
                85   90   95

Glu Asp Leu His His Tyr Arg Asn Leu Ser Xaa Phe Xaa Arg Arg Lys
            100   105   110

Leu Lys Ala Xaa Gly Pro Ala Cys Leu Trp Pro Ala Gln Arg Asp
        115   120   125

<210> 153
<211> 78
<212> PRT
<213> Homo sapiens

<400> 153
Met Ser Pro His Gln Pro Met Gln Val Ser Ser Ser Lys Thr Ile Leu
1   5   10   15

Trp Leu Val Leu Ser Cys Leu Cys Pro Ser Ser Pro His Pro Val Ile
            20   25   30

Ser Gly Leu Pro Gln Trp Tyr Ile Gly Val Leu Ala Gly Ile Val Pro
        35   40   45

Val Ala Pro Ile Arg Pro Gly Asp Ser Gly Leu Asp Leu Gln Arg Glu
    50   55   60

Gly Pro Gln Pro Ile Leu Ser Gln Gly Leu Asn Arg Arg Thr
65   70   75

<210> 154
<211> 245
<212> PRT
<213> Homo sapiens

<400> 154
Met Glu Gly Pro Arg Gly Trp Leu Val Leu Cys Val Leu Ala Ile Ser
1   5   10   15

Leu Ala Ser Met Val Thr Glu Asp Leu Cys Arg Ala Pro Asp Gly Lys
            20   25   30

Lys Gly Glu Ala Gly Arg Pro Gly Arg Arg Gly Arg Pro Gly Leu Lys
35 40 45

Gly Glu Gln Gly Glu Pro Gly Ala Pro Gly Ile Arg Thr Gly Ile Gln
50 55 60

Gly Leu Lys Gly Asp Gln Gly Glu Pro Gly Pro Ser Gly Asn Pro Gly
65 70 75 80

Lys Val Gly Tyr Pro Gly Pro Ser Gly Pro Leu Gly Ala Arg Gly Ile
85 90 95

Pro Gly Ile Lys Gly Thr Lys Gly Ser Pro Gly Asn Ile Lys Asp Gln
100 105 110

Pro Arg Pro Ala Phe Ser Ala Ile Arg Arg Asn Pro Pro Met Gly Gly
115 120 125

Asn Val Val Ile Phe Asp Thr Val Ile Thr Asn Gln Glu Glu Pro Tyr
130 135 140

Gln Asn His Ser Gly Arg Phe Val Cys Thr Val Pro Gly Tyr Tyr Tyr
145 150 155 160

Phe Thr Phe Gln Val Leu Ser Gln Trp Glu Ile Cys Leu Ser Ile Val
165 170 175

Ser Ser Ser Arg Gly Gln Val Arg Arg Ser Leu Gly Phe Cys Asp Thr
180 185 190

Thr Asn Lys Gly Leu Phe Gln Val Val Ser Gly Gly Met Val Leu Gln
195 200 205

Leu Gln Gln Gly Asp Gln Val Trp Val Glu Lys Asp Pro Lys Lys Gly
210 215 220

His Ile Tyr Gln Gly Ser Glu Ala Asp Ser Val Phe Ser Gly Phe Leu
225 230 235 240

Ile Phe Pro Ser Ala
245

<210> 155
<211> 194
<212> PRT
<213> Homo sapiens

<400> 155
Ala Arg Leu Gly Arg Val Pro Glu Ser Gln Ser Arg Arg Gly Ala Ala
1 5 10 15

Gly Ala Ala Phe His His Gly Glu Pro Ser Cys Gln Pro Pro His Arg
20 25 30

Lys Met Leu Arg Arg Arg Gly Ser Pro Gly Met Gly Val His Val Gly
35 40 45

Ala Ala Leu Gly Ala Leu Trp Phe Cys Leu Thr Gly Ala Leu Glu Val
50 55 60

Gln Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr
65 70 75 80

Leu Cys Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu
85 90 95

```
Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe
            100                 105                 110

Ala Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu
        115                 120                 125

Phe Leu Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Ser
    130                 135                 140

Val Arg Val Ala Asp Glu Gly Gln Leu His Leu Leu Arg Glu His Pro
145                 150                 155                 160

Gly Phe Arg Gln Arg Cys Arg Gln Pro Ala Gly Gly Arg Ser Leu Leu
                165                 170                 175

Glu Ala Gln His Asp Pro Gly Ala Gln Gln Gly Pro Ala Ala Arg Gly
            180                 185                 190

Thr Trp


<210> 156
<211> 387
<212> PRT
<213> Homo sapiens

<400> 156
Pro Trp Ser Pro Thr Arg Thr Cys Gly Pro Gly Asp Met Val Thr Ile
  1               5                  10                  15

Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln
             20                  25                  30

Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met
         35                  40                  45

Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Ile Leu Arg Val Val
     50                  55                  60

Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu
 65                  70                  75                  80

Gln Gln Asp Ala His Ser Ser Val Thr Ile Thr Pro Gln Arg Ser Pro
                 85                  90                  95

Thr Gly Ala Val Glu Val Gln Val Pro Glu Asp Pro Val Val Ala Leu
            100                 105                 110

Val Gly Thr Asp Ala Thr Leu His Cys Ser Phe Ser Pro Glu Pro Gly
        115                 120                 125

Phe Ser Leu Thr Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys
    130                 135                 140

Gln Leu Val His Ser Phe Thr Glu Gly Arg Asp Gln Gly Ser Ala Tyr
145                 150                 155                 160

Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala
                165                 170                 175

Ser Leu Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly Ser Phe Thr
            180                 185                 190

Cys Phe Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val Ser Leu Gln
```

195 200 205

Val Ala Ala Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu Pro Asn Lys
210 215 220

Asp Leu Arg Pro Gly Asp Thr Val Thr Ile Thr Cys Ser Ser Tyr Arg
225 230 235 240

Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln Asp Gly Gln Gly Val Pro
245 250 255

Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu Gln Gly Leu
260 265 270

Phe Asp Val His Ser Val Leu Arg Val Val Leu Gly Ala Asn Gly Thr
275 280 285

Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp Ala His Gly
290 295 300

Ser Val Thr Ile Thr Gly Gln Pro Met Thr Phe Pro Pro Glu Ala Leu
305 310 315 320

Trp Val Thr Val Gly Leu Ser Val Cys Leu Ile Ala Leu Leu Val Ala
325 330 335

Leu Pro Phe Val Cys Trp Arg Lys Ile Lys Gln Ser Cys Glu Glu Glu
340 345 350

Asn Ala Gly Ala Glu Asp Gln Asp Gly Glu Gly Glu Gly Ser Lys Thr
355 360 365

Ala Leu Gln Pro Leu Lys His Ser Asp Ser Lys Glu Asp Asp Gly Gln
370 375 380

Glu Ile Ala
385

<210> 157
<211> 30
<212> PRT
<213> Homo sapiens

<400> 157
Pro Pro Glu Ala Leu Trp Val Thr Val Gly Leu Ser Val Cys Leu Ile
1 5 10 15

Ala Leu Leu Val Ala Leu Ala Phe Val Cys Trp Arg Lys Ile
20 25 30

<210> 158
<211> 216
<212> PRT
<213> Homo sapiens

<400> 158
Leu Glu Val Gln Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr
1 5 10 15

Asp Ala Thr Leu Cys Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu
20 25 30

Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val
35 40 45

His Ser Phe Ala Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn Arg
50 55 60

Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg
65 70 75 80

Leu Gln Arg Val Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val
85 90 95

Ser Ile Arg Asp Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala
100 105 110

Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg
115 120 125

Pro Gly Asp Thr Val Thr Ile Thr Cys Ser Ser Tyr Gln Gly Tyr Pro
130 135 140

Glu Ala Glu Val Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly
145 150 155 160

Asn Val Thr Thr Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val
165 170 175

His Ser Ile Leu Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys
180 185 190

Leu Val Arg Asn Pro Val Leu Gln Gln Asp Ala His Ser Ser Val Thr
195 200 205

Ile Thr Gly Gln Pro Met Thr Phe
210 215

<210> 159
<211> 242
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (2)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (5)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (34)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 159
Lys Xaa Pro Cys Xaa Tyr Arg Ser Gly Ile Pro Gly Ser Thr His Ala
1 5 10 15

Ser Val Pro Ser Ala Pro Arg Pro Ser Arg Ala Met Leu Pro Trp Thr
20 25 30

Ala Xaa Gly Leu Ala Leu Ser Leu Arg Leu Ala Leu Ala Arg Ser Gly
35 40 45

Ala Glu Arg Gly Pro Pro Ala Ser Ala Pro Arg Gly Asp Leu Met Phe

```
     50                  55                  60

Leu Leu Asp Ser Ser Ala Ser Val Ser His Tyr Glu Phe Ser Arg Val
 65                 70                  75                  80

Arg Glu Phe Val Gly Gln Leu Val Ala Pro Leu Pro Leu Gly Thr Gly
                85                  90                  95

Ala Leu Arg Ala Ser Leu Val His Val Gly Ser Arg Pro Tyr Thr Glu
            100                 105                 110

Phe Pro Phe Gly Gln His Ser Ser Gly Glu Ala Ala Gln Asp Ala Val
        115                 120                 125

Arg Ala Ser Ala Gln Arg Met Gly Asp Thr His Thr Gly Leu Ala Leu
    130                 135                 140

Val Tyr Ala Lys Glu Gln Leu Phe Ala Glu Ala Ser Gly Ala Arg Pro
145                 150                 155                 160

Gly Val Pro Lys Val Leu Val Trp Val Thr Asp Gly Gly Ser Ser Asp
                165                 170                 175

Pro Val Gly Pro Pro Met Gln Glu Leu Lys Asp Leu Gly Val Thr Val
            180                 185                 190

Phe Ile Val Ser Thr Gly Arg Gly Asn Phe Leu Glu Leu Ser Ala Ala
        195                 200                 205

Ala Ser Ala Pro Ala Glu Lys His Leu His Phe Val Asp Val Asp Asp
    210                 215                 220

Leu His Ile Ile Val Gln Glu Leu Arg Gly Ser Ile Leu Asp Ala Met
225                 230                 235                 240

Arg Pro


<210> 160
<211> 186
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (152)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (180)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (184)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (186)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 160
Ala Pro Ala Trp Gly Gly Pro Gln Gly Arg Trp Ser Arg His Leu Ser
```

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 1               5                   10                  15
Pro Thr Pro Ala Leu Trp Ala Pro Leu Ala Gly His Leu Met Leu Gln
            20                  25                  30
Gln Thr Ala Val Pro Trp His Arg Pro Ala Pro Gly Gln Cys Gly Cys
        35                  40                  45
His Pro Cys Ala Gly Gln Lys His Ala Pro His Pro Gly Gln Pro His
    50                  55                  60
Pro Ser Cys Ala Gly Arg Arg Gly Thr Arg Cys Met Ala Asp Cys Pro
65                  70                  75                  80
Arg Ala Pro Asp Trp His Ala Gly Pro Arg Cys Pro Gly Ala Val Glu
                85                  90                  95
Pro Pro Ala Ala Pro Gln Thr Pro Glu Pro Gly Arg Thr Arg Ser Glu
            100                 105                 110
Arg Arg Trp Leu Ser Cys Pro Ala Gly Thr Ser Gly Pro Leu Gly Gly
        115                 120                 125
Leu Met Leu Val Asp Arg Ala Pro Arg Arg Ser Ala Pro Ala Pro Ala
    130                 135                 140
Ala Ser Ser Gly Pro Gly Arg Xaa Pro Ser Arg Gly Ala Ser Arg Ala
145                 150                 155                 160
Arg Asp Gly Ala Arg Ser Ala Arg Thr Arg Gly Ser Thr Arg Glu Phe
                165                 170                 175
Arg Thr Gly Xaa Cys Arg Val Xaa Ser Xaa
            180                 185

<210> 161
<211> 18
<212> PRT
<213> Homo sapiens

<400> 161
Phe Leu Leu Asp Ser Ser Ala Ser Val Ser His Tyr Glu Phe Ser Arg
 1               5                   10                  15
Val Arg


<210> 162
<211> 14
<212> PRT
<213> Homo sapiens

<400> 162
Gly Ala Leu Arg Ala Ser Leu Val His Val Gly Ser Arg Pro
 1               5                   10

<210> 163
<211> 12
<212> PRT
<213> Homo sapiens

<400> 163
Gly Val Pro Lys Val Leu Val Trp Val Thr Asp Gly
```

```
  1               5                   10


<210> 164
<211> 14
<212> PRT
<213> Homo sapiens

<400> 164
Val Gly Pro Pro Met Gln Glu Leu Lys Asp Leu Gly Val Thr
  1               5                   10


<210> 165
<211> 226
<212> PRT
<213> Homo sapiens

<400> 165
His Ala Ser Val Pro Ser Ala Pro Arg Pro Ser Arg Ala Met Leu Pro
  1               5                   10                  15

Trp Thr Ala Leu Gly Leu Ala Leu Ser Leu Arg Leu Ala Leu Ala Arg
             20                  25                  30

Ser Gly Ala Glu Arg Gly Pro Pro Ala Ser Ala Pro Arg Gly Asp Leu
         35                  40                  45

Met Phe Leu Leu Asp Ser Ser Ala Ser Val Ser His Tyr Glu Phe Ser
     50                  55                  60

Arg Val Arg Glu Phe Val Gly Gln Leu Val Ala Pro Leu Pro Leu Gly
 65                  70                  75                  80

Thr Gly Ala Leu Arg Ala Ser Leu Val His Val Gly Ser Arg Pro Tyr
                 85                  90                  95

Thr Glu Phe Pro Phe Gly Gln His Ser Ser Gly Glu Ala Ala Gln Asp
            100                 105                 110

Ala Val Arg Ala Ser Ala Gln Arg Met Gly Asp Thr His Thr Gly Leu
        115                 120                 125

Ala Leu Val Tyr Ala Lys Glu Gln Leu Phe Ala Glu Ala Ser Gly Ala
    130                 135                 140

Arg Pro Gly Val Pro Lys Val Leu Val Trp Val Thr Asp Gly Gly Ser
145                 150                 155                 160

Ser Asp Pro Val Gly Pro Pro Met Gln Glu Leu Lys Asp Leu Gly Val
                165                 170                 175

Thr Val Phe Ile Val Ser Thr Gly Arg Gly Asn Phe Leu Glu Leu Ser
            180                 185                 190

Ala Ala Ala Ser Ala Pro Ala Glu Lys His Leu His Phe Val Asp Val
        195                 200                 205

Asp Asp Leu His Ile Ile Val Gln Glu Leu Arg Gly Ser Ile Leu Asp
    210                 215                 220

Ala Met
225


<210> 166
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<211> 22
<212> PRT
<213> Homo sapiens

<400> 166
Cys Leu Ile Cys Leu Leu Thr Phe Ile Phe His His Cys Asn His Cys
1 5 10 15

His Glu Glu His Asp His
20

<210> 167
<211> 22
<212> PRT
<213> Homo sapiens

<400> 167
Leu Leu Thr Phe Ile Phe His His Cys Asn His Cys His Glu Glu His
1 5 10 15

Asp His Gly Pro Glu Ala
20

<210> 168
<211> 231
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (2)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 168
Tyr Xaa Lys Val Arg Leu Gln Val Pro Val Arg Asn Ser Arg Val Asp
1 5 10 15

Pro Arg Val Arg Ala Glu Val Leu Arg Ala Thr Arg Gly Gly Ala Ala
20 25 30

Arg Gly Asn Ala Ala Pro Gly Arg Ala Leu Glu Met Val Pro Gly Ala
35 40 45

Ala Gly Trp Cys Cys Leu Val Leu Trp Leu Pro Ala Cys Val Ala Ala
50 55 60

His Gly Phe Arg Ile His Asp Tyr Leu Tyr Phe Gln Val Leu Ser Pro
65 70 75 80

Gly Asp Ile Arg Tyr Ile Phe Thr Ala Thr Pro Ala Lys Asp Phe Gly
85 90 95

Gly Ile Phe His Thr Arg Tyr Glu Gln Ile His Leu Val Pro Ala Glu
100 105 110

Pro Pro Glu Ala Cys Gly Glu Leu Ser Asn Gly Phe Phe Ile Gln Asp
115 120 125

Gln Ile Ala Leu Val Glu Arg Gly Gly Cys Ser Phe Leu Ser Lys Thr
130 135 140

Arg Val Val Gln Glu His Gly Gly Arg Ala Val Ile Ile Ser Asp Asn
145 150 155 160

```
Ala Val Asp Asn Asp Ser Phe Tyr Val Glu Met Ile Gln Asp Ser Thr
                165                 170                 175

Gln Arg Thr Ala Asp Ile Pro Ala Leu Phe Leu Leu Gly Arg Asp Gly
            180                 185                 190

Tyr Met Ile Arg Arg Ser Leu Glu Gln His Gly Leu Pro Trp Ala Ile
        195                 200                 205

Ile Ser Ile Pro Val Asn Val Thr Ser Ile Pro Thr Phe Glu Leu Leu
    210                 215                 220

Gln Pro Pro Trp Thr Phe Trp
225                 230


<210> 169
<211> 261
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (225)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 169
His Glu Leu Lys Met Asp Ala Glu Tyr Ser Gly Asn Glu Phe Pro Arg
  1               5                  10                  15

Ser Glu Gly Glu Arg Asp Gln His Gln Arg Pro Gly Lys Glu Arg Lys
             20                  25                  30

Ser Gly Glu Ala Gly Arg Gly Thr Gly Glu Leu Gly Gln Asp Gly Arg
         35                  40                  45

Leu Leu Ser Ser Thr Leu Ser Leu Ser Ser Asn Arg Ser Leu Gly Gln
     50                  55                  60

Arg Gln Asn Ser Pro Leu Pro Phe Gln Trp Arg Ile Thr His Ser Phe
 65                  70                  75                  80

Arg Trp Met Ala Gln Val Leu Ala Ser Glu Leu Ser Leu Val Ala Phe
                 85                  90                  95

Ile Leu Leu Leu Val Met Ala Phe Ser Lys Lys Trp Leu Asp Leu Ser
            100                 105                 110

Arg Ser Leu Phe Tyr Gln Arg Trp Pro Val Asp Val Ser Asn Arg Ile
        115                 120                 125

His Thr Ser Ala His Val Met Ser Met Gly Leu Leu His Phe Cys Lys
    130                 135                 140

Ser Arg Ser Cys Ser Asp Leu Glu Asn Gly Lys Val Thr Phe Ile Phe
145                 150                 155                 160

Ser Thr Leu Met Leu Phe Pro Ile Asn Ile Trp Ile Phe Glu Leu Glu
                165                 170                 175

Arg Asn Val Ser Ile Pro Ile Gly Trp Ser Tyr Phe Ile Gly Trp Leu
            180                 185                 190

Val Leu Ile Leu Tyr Phe Thr Cys Ala Ile Leu Cys Tyr Phe Asn His
        195                 200                 205
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```
Lys Ser Phe Trp Ser Leu Ile Leu Ser His Pro Ser Gly Ala Val Ser
    210                 215                 220

Xaa Ser Ser Ser Phe Gly Ser Val Glu Glu Ser Pro Arg Ala Gln Thr
225                 230                 235                 240

Ile Thr Asp Thr Pro Ile Thr Gln Glu Gly Val Leu Asp Pro Glu Gln
                245                 250                 255

Lys Asp Thr His Val
            260


<210> 170
<211> 151
<212> PRT
<213> Homo sapiens

<400> 170
Gly Thr Ser Ser Arg Trp Met Gln Ser Thr Leu Gly Met Ser Ser Pro
 1               5                  10                  15

Gly Gln Lys Glu Lys Glu Thr Asn Ile Arg Asp Leu Glu Arg Lys Gly
            20                  25                  30

Arg Val Gly Arg Gln Asp Gly Ala Gln Val Ser Trp Asp Lys Met Gly
        35                  40                  45

Asp Cys Cys Pro Pro Pro Ser Pro Ser Val Val Thr Gly Pro Trp Ala
    50                  55                  60

Ser Ala Arg Thr Leu Arg Cys Pro Phe Asn Gly Glu Ser His Thr Ala
65                  70                  75                  80

Ser Ala Gly Trp Pro Arg Cys Trp Pro Leu Ser Ser Ala Trp Leu Pro
                85                  90                  95

Leu Ser Tyr Tyr Trp Ser Trp Pro Ser Pro Arg Asn Gly Trp Thr Ser
            100                 105                 110

Leu Gly Ala Ser Ser Thr Ser Ala Gly Pro Trp Met Ser Ala Thr Glu
        115                 120                 125

Ser Thr His Gln Pro Thr Leu Cys Pro Trp Gly Ser Cys Thr Phe Ala
    130                 135                 140

Asn Pro Gly Ala Val Leu Thr
145                 150


<210> 171
<211> 317
<212> PRT
<213> Homo sapiens

<400> 171
Ala Arg Ala Glu Val Ile Leu Cys Thr Lys Glu Val Ser Val Gly Ala
 1               5                  10                  15

Arg Lys Asn Ala Phe Ala Leu Leu Val Glu Met Gly His Ala Phe Leu
            20                  25                  30

Arg Phe Gly Ser Asn Gln Glu Glu Ala Leu Gln Cys Tyr Leu Val Leu
        35                  40                  45

Ile Tyr Pro Gly Leu Val Gly Ala Val Thr Met Val Ser Cys Ser Ile
```

```
    50                  55                  60

Leu Ala Leu Thr His Leu Leu Phe Glu Phe Lys Gly Leu Met Gly Thr
 65                  70                  75                  80

Ser Thr Val Glu Gln Leu Leu Glu Asn Val Cys Leu Leu Leu Ala Ser
                 85                  90                  95

Arg Thr Arg Asp Val Val Lys Ser Ala Leu Gly Phe Ile Lys Val Ala
            100                 105                 110

Val Thr Val Met Asp Val Ala His Leu Ala Lys His Val Gln Leu Val
        115                 120                 125

Met Glu Ala Ile Gly Lys Leu Ser Asp Asp Met Arg Arg His Phe Arg
    130                 135                 140

Met Lys Leu Arg Asn Leu Phe Thr Lys Phe Ile Arg Lys Phe Gly Phe
145                 150                 155                 160

Glu Leu Val Lys Arg Leu Leu Pro Glu Glu Tyr His Arg Val Leu Val
                165                 170                 175

Asn Ile Arg Lys Ala Glu Ala Arg Ala Lys Arg His Arg Ala Leu Ser
            180                 185                 190

Gln Ala Ala Val Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Pro
        195                 200                 205

Ala Gln Gly Lys Gly Asp Ser Ile Glu Glu Ile Leu Ala Asp Ser Glu
    210                 215                 220

Asp Glu Glu Asp Asn Glu Glu Glu Glu Arg Ser Arg Gly Lys Glu Gln
225                 230                 235                 240

Arg Lys Leu Ala Arg Gln Arg Ser Arg Ala Trp Leu Lys Glu Gly Gly
                245                 250                 255

Gly Asp Glu Pro Leu Asn Phe Leu Asp Pro Lys Val Ala Gln Arg Val
            260                 265                 270

Leu Ala Thr Gln Pro Gly Pro Ala Gly Gln Glu Glu Gly Pro Gln Leu
        275                 280                 285

Gln Gly Glu Arg Arg Trp Pro Ala Asp His Lys Gly Gly Gly Arg Arg
    290                 295                 300

Gln Gln Asp Gly Gly Arg Gly Arg Cys Gln Arg Arg Arg
305                 310                 315


<210> 172
<211> 167
<212> PRT
<213> Homo sapiens

<400> 172
Gly Thr Arg Glu Gly Glu Gly Arg Lys Cys Pro Trp Lys Gly Leu Arg
  1               5                  10                  15

Ala Arg Thr Gly Met Gly Gln Glu Val His Gly Ser Cys Trp Ala Leu
             20                  25                  30

Gly Ala Gly Gly Gly Gln Arg Gln Trp Val Gly Arg Ser Met Pro Pro
         35                  40                  45
```

Leu Ala Pro Gln Leu Cys Arg Ala Val Phe Leu Val Pro Ile Leu Leu
50 55 60

Leu Leu Gln Val Lys Pro Leu Asn Gly Ser Pro Gly Pro Lys Asp Gly
65 70 75 80

Ser Gln Thr Glu Lys Thr Pro Ser Ala Asp Gln Asn Gln Glu Gln Phe
85 90 95

Glu Glu His Phe Val Ala Ser Ser Val Gly Glu Met Trp Gln Val Val
100 105 110

Asp Met Ala Gln Gln Glu Glu Asp Gln Ser Ser Lys Thr Ala Ala Val
115 120 125

His Lys His Ser Phe His Leu Ser Phe Cys Phe Ser Leu Ala Ser Val
130 135 140

Met Val Phe Ser Gly Gly Pro Leu Arg Arg Thr Phe Pro Asn Ile Gln
145 150 155 160

Leu Cys Phe Met Leu Thr His
165

<210> 173
<211> 267
<212> PRT
<213> Homo sapiens

<400> 173
Met Ala Gly Gly Trp Ala Ala Glu Ala Val Trp Ala Gly Phe Gly Val
1 5 10 15

Val Val Val Ala Arg Arg Leu Val Leu Leu Pro Leu Leu Leu His Pro
20 25 30

Gly Phe Gln Gln Leu Leu Leu Val Leu Leu Leu Pro His Glu Gln Leu
35 40 45

His His Glu His Leu Leu Leu Val Asp Leu Leu Ala Asp Val Leu Gly
50 55 60

Asp Val Arg Asp Asp Pro Val His Lys Val Ala His Glu His Asp Gln
65 70 75 80

Val Leu Glu Asp Asp Asp Lys Arg Gln Pro Gly Cys Gln Asp Gly Pro
85 90 95

Glu Val Leu Gly Asp Val Val Leu Val Phe Arg Pro Arg Arg Leu Ser
100 105 110

Val Val Phe Ile Pro Ala Asp Leu His Leu Val Ala Gln Val Gln Gly
115 120 125

Val Ile Gly Gly Arg Ala Val Leu Glu Val Thr Asp Val Glu Gly Gly
130 135 140

Glu Gly Val Val Asp Glu Ala Val His Gly Pro Val Leu Thr Val His
145 150 155 160

Val Glu Val His Gln Ala Arg Asp Glu Val Arg Arg Glu Gly Asp His
165 170 175

Glu Gly Ile Asp Asp Asp Ser Lys Leu Pro Asn Ala Ser Glu Asp Ile
180 185 190

```
Val Pro Asp Ser Asp Val Phe Gly Ser Asp Ser Tyr Arg Pro Ser Glu
        195                 200                 205

Leu Ser Asp Lys Leu Phe Gly Val Gln Ala Asp Leu Asp Asp Val Val
    210                 215                 220

Gln Gln Arg Lys Gln Trp Gly Gln Gly Glu Gly Gly Asp Lys Gln Gly
225                 230                 235                 240

Asp Glu Ala Lys Leu Asp Asp His Phe His Val Leu Trp Gly Glu Ala
                245                 250                 255

Arg Glu Gly Leu Gln Val Val Ile His Leu Val
            260                 265
```

<210> 174
<211> 194
<212> PRT
<213> Homo sapiens

<400> 174

```
Pro Arg Ala Ala Gly Ile Arg His Glu Leu Ile His Gly Leu Trp Asn
  1               5                  10                  15

Leu Val Phe Leu Phe Ser Asn Leu Ser Leu Ile Phe Leu Met Pro Phe
             20                  25                  30

Ala Tyr Phe Phe Thr Glu Ser Glu Gly Phe Ala Gly Ser Arg Lys Gly
         35                  40                  45

Val Leu Gly Arg Val Tyr Glu Thr Val Val Met Leu Met Leu Leu Thr
     50                  55                  60

Leu Leu Val Leu Gly Met Val Trp Val Ala Ser Ala Ile Val Asp Lys
 65                  70                  75                  80

Asn Lys Ala Asn Arg Glu Ser Leu Tyr Asp Phe Trp Glu Tyr Tyr Leu
                 85                  90                  95

Pro Tyr Leu Tyr Ser Cys Ile Ser Phe Leu Gly Val Leu Leu Leu Leu
            100                 105                 110

Gly Glu Cys Thr Gly Ser Gly Arg Glu Trp Ala Gly Ser Leu Asp Gln
        115                 120                 125

Ser Asn Gln Ala Arg Arg Lys Gly Asn Gly Gly His Val Arg Glu Gly
    130                 135                 140

Val Glu Ser Arg Val Trp Gln Val Thr Gly Ser Cys Pro Tyr Ser Val
145                 150                 155                 160

Tyr Ser Thr Gly Ser Arg Pro His Val Leu Arg His Trp Glu Ala Ala
                165                 170                 175

Ser Gln Ala Pro Ala Ala Gly Arg Pro Gly Gly Ala Ala Val Leu Leu
            180                 185                 190

Ser Leu
```

<210> 175
<211> 171
<212> PRT

<213> Homo sapiens

<400> 175
His Ala Ser Ala Phe Phe Gly Thr Arg Ala Leu Leu Ser Val Ser Leu
1 5 10 15

Pro Pro Pro Cys Met Leu His Trp Val Leu Ser Phe Phe Phe Leu Leu
20 25 30

Ser Cys Pro Arg Thr Glu Gly Leu Pro Gly Leu Tyr Cys Pro Gly Cys
35 40 45

Ser Gln Cys Pro Gly Arg Gly Met Trp Pro Gly Asp Pro Gly Pro Gly
50 55 60

Ile Gln Gly Pro Gly Leu Asp Leu Arg Thr Gly Met Glu Ala Thr Gly
65 70 75 80

Ala Gln Gln Pro Thr Leu Ser Ser Pro His Cys Leu Leu Ser Leu Pro
85 90 95

Thr Leu Pro Ala Arg Ala Val Gln Leu Arg Trp Asp Leu Ser Ile Ser
100 105 110

Arg Ala Gly Gly Arg Val Ala Val Leu Gly Leu Cys Leu Glu Pro Gly
115 120 125

Gly Ser Leu Leu Leu Pro Pro Ser Ala Leu Pro Glu Thr Asp Pro Cys
130 135 140

Ala Ala Cys Pro Pro Cys Pro Phe Val Pro Met Ser Gly Gly Gly Gly
145 150 155 160

Arg Pro Thr Val Pro Glu Ala Gly His Gln Pro
165 170

<210> 176
<211> 132
<212> PRT
<213> Homo sapiens

<400> 176
Ser His Thr Arg Pro Thr Glu Gln Pro Ser Val Leu Pro Leu Phe Met
1 5 10 15

Met Tyr Val Met Met Ala Tyr Leu Thr Leu Phe Gln Met Gly Ser Trp
20 25 30

Met Ser Phe Ser Leu Ser Leu Cys Ser Leu Leu Phe Ile Leu Thr Gly
35 40 45

His Cys Leu Ser Glu Asn Phe Tyr Val Arg Gly Asp Gly Thr Arg Ala
50 55 60

Tyr Phe Phe Thr Lys Gly Glu Val His Ser Met Phe Cys Lys Ala Ser
65 70 75 80

Leu Asp Glu Lys Gln Asn Leu Val Asp Arg Arg Leu Gln Val Asn Arg
85 90 95

Lys Lys Gln Val Lys Met His Arg Val Trp Ile Gln Gly Lys Phe Gln
100 105 110

Lys Pro Leu His Gln Thr Gln Asn Ser Ser Asn Met Val Ser Thr Leu
115 120 125

```
Leu Ser Gln Asp
    130


<210> 177
<211> 167
<212> PRT
<213> Homo sapiens

<400> 177
Ala Arg Glu Ser Ser Trp Asp His Val Lys Thr Ser Ala Thr Asn Arg
  1               5                  10                  15

Phe Ser Arg Met His Cys Pro Thr Val Pro Asp Glu Lys Asn His Tyr
             20                  25                  30

Glu Lys Ser Ser Gly Ser Ser Glu Gly Gln Ser Lys Thr Glu Ser Asp
         35                  40                  45

Phe Ser Asn Leu Asp Ser Glu Lys His Lys Lys Gly Pro Met Glu Thr
     50                  55                  60

Gly Leu Phe Pro Gly Ser Asn Ala Thr Phe Arg Ile Leu Glu Val Gly
 65                  70                  75                  80

Cys Gly Ala Gly Asn Ser Val Phe Pro Ile Leu Asn Thr Leu Glu Asn
                 85                  90                  95

Ser Pro Glu Ser Phe Leu Tyr Cys Cys Asp Phe Ala Ser Gly Ala Val
            100                 105                 110

Glu Leu Val Lys Ser His Ser Ser Tyr Arg Ala Thr Gln Cys Phe Ala
        115                 120                 125

Phe Val His Asp Val Cys Asp Asp Gly Leu Pro Tyr Pro Phe Pro Asp
    130                 135                 140

Gly Ile Leu Asp Val Ile Leu Leu Val Phe Val Leu Ser Ser Ile His
145                 150                 155                 160

Pro Asp Arg Thr Leu Phe Ile
                165


<210> 178
<211> 116
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (66)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (104)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 178
His Glu Gln Glu Pro Leu Pro Ala Pro Val Ala Glu Ala Ala Leu Pro
  1               5                  10                  15

Ser Ala Arg Asn Ser Ser Val Leu Ala Ser Leu Ser Pro His Thr Gly
             20                  25                  30
```

```
Pro Ala Gly Leu Leu Arg Asp Ser Ser Val Gln Val Ser Thr Leu Gly
        35                  40                  45

Cys Leu Leu Gly Cys Gly Gly Arg Met Phe Phe Pro Cys Leu Pro Thr
    50                  55                  60

Leu Xaa Leu Arg Ile Leu His Ser Gly Trp Val Gly Leu Phe Leu Leu
 65                  70                  75                  80

Ile Ser Ser Arg Ala Pro Ser Ser Ser Leu Ala Trp Lys His Gly Pro
                85                  90                  95

Gly Glu Leu Trp Trp Pro Arg Xaa Pro Leu Arg Ser Cys Thr Gly Leu
            100                 105                 110

Ala Ser Cys Gly
        115


<210> 179
<211> 189
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (130)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (166)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (189)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 179
Leu Thr Pro Ala Leu Pro Ser Pro Arg Ser Ala Ser Pro Leu Leu Ser
  1               5                  10                  15

Pro Glu Ser Leu Gln Ser Pro Gln Trp Pro Ser Ser Ser Leu Ser Ile
             20                  25                  30

His Ser Leu Pro Val Ala Gly Lys Pro Ser Leu Ile Thr Ser Leu Phe
        35                  40                  45

Thr Glu Pro Cys Asp Gly Phe Met Ala Ile Arg Gly Ser Asn Thr Gln
    50                  55                  60

Gly Leu Thr Met Met Thr Met Thr Ser Asp Arg Trp Phe Ser Met Ala
 65                  70                  75                  80

Trp Ala Ser Cys Ser Leu Ser Arg Pro Pro Leu Thr Pro Ser Cys Ser
                85                  90                  95

Cys Gln Gln Pro Ala Thr Val Ala Leu Leu Leu Gln Thr Ile Ser Val
            100                 105                 110

Cys Ser Ala Gln Gln Ala Asp Pro Leu Ser Pro Pro Arg Ala Cys Arg
        115                 120                 125

Pro Xaa Arg Gln Phe Pro Val Leu Gln Ser Ala Gly Pro Pro His Ser
```

130 135 140

Pro His Val Tyr Ala Phe Val Leu Phe Pro Val Ser Ser Arg Trp Gln
145 150 155 160

Gly Gly Asp Phe Cys Xaa Ile Cys Cys Cys Phe Pro Gln Cys Leu Gly
165 170 175

Arg Cys Leu Glu His Thr Arg Cys Ser Ile Asn Pro Xaa
180 185

<210> 180
<211> 98
<212> PRT
<213> Homo sapiens

<400> 180
Arg Leu Cys Arg Glu Thr Ala Leu Met Ser Leu Cys Leu Val Leu Met
1 5 10 15

Arg Arg Met Gly Trp Ile Asp Leu Leu Leu Pro Glu Leu Gly Ala Leu
20 25 30

Arg Val Phe Leu His Leu Phe Leu Val Ala Leu Arg Thr Lys Arg Trp
35 40 45

Ile Phe Arg Thr Leu Gly Gln Leu Thr Cys Val Asn Ile Leu Gly Asp
50 55 60

Ser Arg Lys Lys Arg Glu Cys Arg Leu Asn Lys Arg Gln Leu Gln Phe
65 70 75 80

Gly Glu Lys Thr Leu Gln Val Pro Glu Arg Leu Val Val Arg His Ser
85 90 95

Pro Phe

<210> 181
<211> 310
<212> PRT
<213> Homo sapiens

<400> 181
Met Leu Leu Pro Phe Ile Lys Leu Pro Thr Thr Gly Asn Ser Leu Ala
1 5 10 15

Lys Ile Gln Thr Val Gly Gln Asn Gln Gln Lys Val Asn Arg Val Leu
20 25 30

Met Gly Pro Arg Ser Ile Gln Lys Arg His Phe Lys Glu Val Gly Arg
35 40 45

Gln Ser Ile Arg Arg Glu Gln Gly Ala Gln Ala Ser Val Glu Asn Ala
50 55 60

Ala Glu Glu Lys Arg Leu Gly Ser Pro Ala Pro Arg Glu Leu Glu Gln
65 70 75 80

Pro His Thr Gln Gln Gly Pro Glu Lys Leu Ala Gly Asn Ala Ile Tyr
85 90 95

Thr Lys Pro Ser Phe Thr Gln Glu His Lys Ala Ala Val Ser Val Leu
100 105 110

Thr Pro Phe Ser Lys Gly Ala Pro Ser Thr Ser Ser Pro Ala Lys Ala
115 120 125

Leu Pro Gln Val Arg Asp Arg Trp Lys Asp Asn Thr His Thr Ile Ser
130 135 140

Ile Leu Glu Ser Ala Lys Ala Arg Val Thr Asn Met Lys Ala Ser Lys
145 150 155 160

Pro Ile Ser His Ser Arg Lys Lys Tyr Arg Phe His Lys Thr Arg Ser
165 170 175

Arg Met Thr His Arg Thr Pro Lys Val Lys Lys Ser Pro Lys Phe Arg
180 185 190

Lys Lys Ser Tyr Leu Ser Arg Leu Met Leu Ala Asn Arg Pro Pro Phe
195 200 205

Ser Ala Ala Lys Ser Leu Ile Asn Ser Pro Ser Gln Gly Ala Phe Ser
210 215 220

Ser Leu Gly Asp Leu Ser Pro Gln Glu Asn Pro Phe Leu Glu Val Ser
225 230 235 240

Ala Pro Ser Glu His Phe Ile Glu Thr Thr Asn Ile Lys Asp Thr Thr
245 250 255

Ala Arg Asn Ala Leu Glu Glu Asn Val Phe Met Glu Asn Thr Asn Met
260 265 270

Pro Glu Val Thr Ile Ser Glu Asn Thr Asn Tyr Asn His Pro Pro Glu
275 280 285

Ala Asp Ser Ala Gly Thr Ala Phe Asn Leu Gly Pro Thr Val Lys Gln
290 295 300

Thr Glu Thr Asn Ser Cys
305 310

<210> 182
<211> 139
<212> PRT
<213> Homo sapiens

<400> 182
Leu Lys Glu Met Ala Glu Leu His His Gly Arg Ser Thr Ser Leu Cys
1 5 10 15

Ile Leu Pro Leu Gln Arg Thr Arg Ile His Ser Met Ser Ala Ser Leu
20 25 30

Trp Cys Phe Arg Ser Gln Gln Ser Ile Pro Met Arg Cys His Arg Ser
35 40 45

Leu Ser Glu Ile Pro Glu Asp Phe Gln Met Asn Arg Ser Thr Arg Ser
50 55 60

Tyr Arg Cys Trp Ala Thr Trp Pro Arg Leu Gly Trp Ala Leu Pro Cys
65 70 75 80

Cys Met Asn Ser Leu Arg Lys Gly Arg Lys Phe Ser Gln Ile Thr Thr
85 90 95

Ser Leu Met Ala Ser Val Ser Ser Ala Ser Met Val Ser Arg Arg Arg

```
            100                 105                 110

Arg Pro Leu Pro Lys His Pro Val Thr Thr Thr Ser Thr Ala Thr Ala
        115                 120                 125

Leu Leu Gly Thr Ser Ser Thr Trp Ser Lys Ser
    130                 135


<210> 183
<211> 103
<212> PRT
<213> Homo sapiens

<400> 183
Thr Arg Pro Asp Trp Val Leu Pro Ser Glu Val Glu Val Leu Glu Ser
  1               5                  10                  15

Ile Tyr Leu Asp Glu Leu Gln Val Ile Lys Gly Asn Gly Arg Thr Ser
             20                  25                  30

Pro Trp Glu Ile Tyr Ile Thr Leu His Pro Ala Thr Ala Glu Asp Gln
         35                  40                  45

Asp Ser Gln Tyr Val Cys Phe Thr Leu Val Leu Gln Val Pro Ala Glu
     50                  55                  60

Tyr Pro His Glu Val Pro Gln Ile Ser Ile Arg Asn Pro Arg Gly Leu
 65                  70                  75                  80

Ser Asp Glu Gln Ile His Thr Ile Leu Gln Val Leu Gly His Val Ala
                 85                  90                  95

Lys Ala Gly Leu Gly Thr Ala
            100


<210> 184
<211> 347
<212> PRT
<213> Homo sapiens

<400> 184
Met Leu Tyr Glu Leu Ile Glu Lys Gly Lys Glu Ile Leu Thr Asp Asn
  1               5                  10                  15

Asn Ile Pro His Gly Gln Cys Val Ile Cys Leu Tyr Gly Phe Gln Glu
             20                  25                  30

Lys Glu Ala Phe Thr Lys Thr Pro Cys Tyr His Tyr Phe His Cys His
         35                  40                  45

Cys Leu Ala Arg Tyr Ile Gln His Met Glu Gln Glu Leu Lys Ala Gln
     50                  55                  60

Gly Gln Glu Gln Glu Gln Glu Arg Gln His Ala Thr Thr Lys Gln Lys
 65                  70                  75                  80

Ala Val Gly Val Gln Cys Pro Val Cys Arg Glu Pro Leu Val Tyr Asp
                 85                  90                  95

Leu Ala Ser Leu Lys Ala Ala Pro Glu Pro Gln Gln Pro Met Glu Leu
            100                 105                 110

Tyr Gln Pro Ser Ala Glu Ser Leu Arg Gln Gln Glu Glu Arg Lys Arg
        115                 120                 125
```

Leu Tyr Gln Arg Gln Gln Glu Arg Gly Gly Ile Ile Asp Leu Glu Ala
130 135 140

Glu Arg Asn Arg Tyr Phe Ile Ser Leu Gln Gln Pro Pro Ala Pro Ala
145 150 155 160

Glu Pro Glu Ser Ala Val Asp Val Ser Lys Gly Ser Gln Pro Pro Ser
165 170 175

Thr Leu Ala Ala Glu Leu Ser Thr Ser Pro Ala Val Gln Ser Thr Leu
180 185 190

Pro Pro Pro Leu Pro Val Ala Thr Gln His Ile Cys Glu Lys Ile Pro
195 200 205

Gly Thr Arg Ser Asn Gln Gln Arg Leu Gly Glu Thr Gln Lys Ala Met
210 215 220

Leu Asp Pro Pro Lys Pro Ser Arg Gly Pro Trp Arg Gln Pro Glu Arg
225 230 235 240

Arg His Pro Lys Gly Gly Glu Cys His Ala Pro Lys Gly Thr Arg Asp
245 250 255

Thr Gln Glu Leu Pro Pro Pro Glu Gly Pro Leu Lys Glu Pro Met Asp
260 265 270

Leu Lys Pro Glu Pro His Ser Gln Gly Val Glu Gly Pro Pro Gln Glu
275 280 285

Lys Gly Pro Gly Ser Trp Gln Gly Pro Pro Pro Arg Arg Thr Arg Asp
290 295 300

Cys Val Arg Trp Glu Arg Ser Lys Gly Arg Thr Pro Gly Ser Ser Tyr
305 310 315 320

Pro Arg Leu Pro Arg Gly Gln Gly Ala Tyr Arg Pro Gly Thr Arg Arg
325 330 335

Glu Ser Leu Gly Leu Glu Ser Lys Asp Gly Ser
340 345

<210> 185
<211> 147
<212> PRT
<213> Homo sapiens

<400> 185
His Asp Thr Arg Leu Pro Leu Pro Gly Gln His Gly Arg Gly Ala Trp
1 5 10 15

Val Cys Leu Thr Val Leu Val Cys Ser Thr Val Asp Ser Asn Asp Ser
20 25 30

Leu Tyr Gly Gly Asp Ser Lys Phe Leu Ala Glu Asn Asn Lys Leu Cys
35 40 45

Glu Thr Val Met Ala Gln Ile Leu Glu His Leu Lys Thr Leu Ala Lys
50 55 60

Asp Glu Ala Leu Lys Arg Gln Ser Ser Leu Gly Leu Ser Phe Phe Asn
65 70 75 80

Ser Ile Leu Ala His Gly Asp Leu Arg Asn Asn Lys Leu Asn Gln Leu

85 90 95

Ser Val Asn Leu Trp His Leu Ala Gln Arg His Gly Cys Ala Asp Thr
100 105 110

Arg Thr Met Val Lys Thr Leu Glu Tyr Ile Lys Lys Gln Ser Lys Gln
115 120 125

Pro Asp Met Thr His Leu Thr Glu Leu Ala Leu Arg Leu Pro Leu Gln
130 135 140

Thr Arg Thr
145

<210> 186
<211> 75
<212> PRT
<213> Homo sapiens

<400> 186
Met Leu Phe Val Asp Ser Gly Ser Thr Arg Leu Arg Lys Lys Thr Leu
1 5 10 15

Ser Gly Asp Phe Ile Phe Met Asn Arg Cys Gln Ser Ser Arg Gln Pro
20 25 30

Arg Pro Ala Gly Val Asn Lys His Leu Trp Gly Cys Pro Ala Ser Ser
35 40 45

Arg Thr Ser His Glu Trp Leu Leu Trp Pro Lys Ala Val Leu Gln Ala
50 55 60

Lys Gln Thr Ala Leu Gly Trp Asn Ser Pro Thr
65 70 75

<210> 187
<211> 50
<212> PRT
<213> Homo sapiens

<400> 187
Cys Gln Ser Ser Arg Gln Pro Arg Pro Ala Gly Val Asn Lys His Leu
1 5 10 15

Trp Gly Cys Pro Ala Ser Ser Arg Thr Ser His Glu Trp Leu Leu Trp
20 25 30

Pro Lys Ala Val Leu Gln Ala Lys Gln Thr Ala Leu Gly Trp Asn Ser
35 40 45

Pro Thr
50

<210> 188
<211> 33
<212> PRT
<213> Homo sapiens

<400> 188
Lys Trp Gly Cys Phe Cys Lys Gly Ser Ser Phe Thr Pro His Ser Cys
1 5 10 15

Pro Pro Glu Ala Pro Leu Phe Pro Ala Val Leu Leu Val Ser Thr Leu

20 25 30

Gly

<210> 189
<211> 18
<212> PRT
<213> Homo sapiens

<400> 189
Cys Pro Pro Glu Ala Pro Leu Phe Pro Ala Val Leu Leu Val Ser Thr
1 5 10 15

Leu Gly

<210> 190
<211> 154
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (152)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 190
Glu Gly Ala Asp Lys Met Ala Thr Ser Val Gly His Arg Cys Leu Gly
1 5 10 15

Leu Leu His Gly Val Ala Pro Trp Arg Ser Ser Leu His Pro Cys Glu
20 25 30

Ile Thr Ala Leu Ser Gln Ser Leu Gln Pro Leu Arg Lys Leu Pro Phe
35 40 45

Arg Ala Phe Arg Thr Asp Ala Arg Lys Ile His Thr Ala Pro Ala Arg
50 55 60

Thr Met Phe Leu Leu Arg Pro Leu Pro Ile Leu Leu Val Thr Gly Gly
65 70 75 80

Gly Tyr Ala Gly Tyr Arg Gln Tyr Glu Lys Tyr Arg Glu Arg Glu Leu
85 90 95

Glu Lys Leu Gly Leu Glu Ile Pro Pro Lys Leu Ala Gly His Trp Glu
100 105 110

Val Ala Leu Tyr Lys Ser Val Pro Thr Arg Leu Leu Ser Arg Ala Trp
115 120 125

Gly Arg Leu Asn Gln Val Glu Leu Pro His Trp Leu Arg Arg Pro Val
130 135 140

Tyr Ser Leu Tyr Ile Trp Thr Xaa Gly Gly
145 150

<210> 191
<211> 142
<212> PRT
<213> Homo sapiens

<400> 191
Arg Glu Gln Leu Ser Cys Phe Ser Ser His Thr Trp Cys Pro Trp Glu
1   5   10   15

Gly Val Leu Trp Ala Pro Gln Ala Gln Gly Val Met Ser Ala Pro Pro
            20   25   30

Pro His Pro Gln Pro Pro Ala Ala Pro Thr Ser Arg Asn Tyr Thr Glu
        35   40   45

Ile Arg Glu Lys Leu Arg Ser Arg Leu Thr Arg Arg Lys Glu Glu Leu
    50   55   60

Pro Met Lys Gly Gly Thr Leu Gly Gly Ile Pro Gly Glu Pro Ala Val
65   70   75   80

Asp His Arg Asp Val Asp Glu Leu Leu Glu Phe Ile Asn Ser Thr Glu
                85   90   95

Pro Lys Val Pro Asn Ser Ala Arg Ala Ala Lys Arg Ala Arg His Lys
            100   105   110

Leu Lys Lys Lys Val Gly Val Gly Arg Ala Gln Leu Cys Arg Leu Ser
        115   120   125

Ser Leu Arg Thr Leu Ala Pro Thr Pro Arg Thr Ser Gly Ala
    130   135   140

<210> 192
<211> 63
<212> PRT
<213> Homo sapiens

<400> 192
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Val Lys Arg Arg Gly Pro Leu Val Arg Val Glu Gln Leu Arg Ile Glu
            20   25   30

Glu Met Lys Val Ile Lys Leu Leu Val Thr Phe Glu Leu Gly Val Ile
        35   40   45

Ile Leu Ile Leu Glu Met Thr Lys Leu Arg Leu Thr Lys Thr Arg
    50   55   60

<210> 193
<211> 218
<212> PRT
<213> Homo sapiens

<400> 193
Thr Leu Leu Lys Gly Thr Lys Leu Glu Leu His Arg Gly Gly Gly Arg
1   5   10   15

Ser Arg Thr Ser Gly Ser Pro Gly Leu Gln Glu Phe Gly Thr Arg Pro
            20   25   30

Thr Pro Gly Val Trp Ser Cys Pro Thr Ala Thr Pro Trp Ala Ser Gly
        35   40   45

Ser Arg Arg Lys Asn Leu Ala Arg Glu Ser Lys Gly Arg Pro Arg Pro
    50   55   60

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Thr Glu Ile Thr Arg Pro Tyr Leu Cys Pro His Pro Tyr Leu Pro Pro
 65                 70                  75                  80

His Thr Ala Pro Cys Leu Gly Ser His Pro Ser Ala Cys Arg Cys Ser
                85                  90                  95

Arg Ser Cys Pro His Ser Leu Leu Leu Pro Phe Ser Ile Thr Arg Glu
            100                 105                 110

Cys Pro Gly Ser His Arg Val Pro Gln Met Pro Val Phe Pro Gln Thr
        115                 120                 125

Ile Leu Ser Ser Arg Ile Asn Ser Ile Ala Ile Gln Met Ser Pro His
    130                 135                 140

Gln Pro Met Gln Val Ser Ser Ser Lys Thr Ile Leu Trp Leu Val Leu
145                 150                 155                 160

Ser Cys Leu Cys Pro Ser Ser Pro His Pro Val Ile Ser Gly Leu Pro
                165                 170                 175

Gln Trp Tyr Ile Gly Val Leu Ala Gly Ile Val Pro Val Ala Pro Ile
            180                 185                 190

Arg Pro Gly Asp Ser Gly Leu Asp Leu Gln Arg Glu Gly Pro Gln Pro
        195                 200                 205

Ile Leu Ser Gln Gly Leu Asn Arg Arg Thr
    210                 215


<210> 194
<211> 704
<212> PRT
<213> Homo sapiens

<400> 194
Val Asp Gly Ala Ala Met Ala Ala Cys Glu Gly Arg Arg Ser Gly Ala
  1               5                  10                  15

Leu Gly Ser Ser Gln Ser Asp Phe Leu Thr Pro Pro Val Gly Gly Ala
            20                  25                  30

Pro Trp Ala Val Ala Thr Thr Val Val Met Tyr Pro Pro Pro Pro Pro
        35                  40                  45

Pro Pro His Arg Asp Phe Ile Ser Val Thr Leu Ser Phe Gly Glu Ser
    50                  55                  60

Tyr Asp Asn Ser Lys Ser Trp Arg Arg Arg Ser Cys Trp Arg Lys Trp
 65                 70                  75                  80

Lys Gln Leu Ser Arg Leu Gln Arg Asn Met Ile Leu Phe Leu Leu Ala
                85                  90                  95

Phe Leu Leu Phe Cys Gly Leu Leu Phe Tyr Ile Asn Leu Ala Asp His
            100                 105                 110

Trp Lys Ala Leu Ala Phe Arg Leu Glu Glu Glu Gln Lys Met Arg Pro
        115                 120                 125

Glu Ile Ala Gly Leu Lys Pro Ala Asn Pro Pro Val Leu Pro Ala Pro
    130                 135                 140

Gln Lys Ala Asp Thr Asp Pro Glu Asn Leu Pro Glu Ile Ser Ser Gln
145                 150                 155                 160
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Lys Thr Gln Arg His Ile Gln Arg Gly Pro Pro His Leu Gln Ile Arg
                165                 170                 175

Pro Pro Ser Gln Asp Leu Lys Asp Gly Thr Gln Glu Glu Ala Thr Lys
            180                 185                 190

Arg Gln Glu Ala Pro Val Asp Pro Arg Pro Glu Gly Asp Pro Gln Arg
        195                 200                 205

Thr Val Ile Ser Trp Arg Gly Ala Val Ile Glu Pro Glu Gln Gly Thr
    210                 215                 220

Glu Leu Pro Ser Arg Arg Ala Glu Val Pro Thr Lys Pro Pro Leu Pro
225                 230                 235                 240

Pro Ala Arg Thr Gln Gly Thr Pro Val His Leu Asn Tyr Arg Gln Lys
                245                 250                 255

Gly Val Ile Asp Val Phe Leu His Ala Trp Lys Gly Tyr Arg Lys Phe
            260                 265                 270

Ala Trp Gly His Asp Glu Leu Lys Pro Val Ser Arg Ser Phe Ser Glu
        275                 280                 285

Trp Phe Gly Leu Gly Leu Thr Leu Ile Asp Ala Leu Asp Thr Met Trp
    290                 295                 300

Ile Leu Gly Leu Arg Lys Glu Phe Glu Glu Ala Arg Lys Trp Val Ser
305                 310                 315                 320

Lys Lys Leu His Phe Glu Lys Asp Val Asp Val Asn Leu Phe Glu Ser
                325                 330                 335

Thr Ile Arg Ile Leu Gly Gly Leu Leu Ser Ala Tyr His Leu Ser Gly
            340                 345                 350

Asp Ser Leu Phe Leu Arg Lys Ala Glu Asp Phe Gly Asn Arg Leu Met
        355                 360                 365

Pro Ala Phe Arg Thr Pro Ser Lys Ile Pro Tyr Ser Asp Val Asn Ile
    370                 375                 380

Gly Thr Gly Val Ala His Pro Pro Arg Trp Thr Ser Asp Ser Thr Val
385                 390                 395                 400

Ala Glu Val Thr Ser Ile Gln Leu Glu Phe Arg Glu Leu Ser Arg Leu
                405                 410                 415

Thr Gly Asp Lys Lys Phe Gln Glu Ala Val Glu Lys Val Thr Gln His
            420                 425                 430

Ile His Gly Leu Ser Gly Lys Lys Asp Gly Leu Val Pro Met Phe Ile
        435                 440                 445

Asn Thr His Ser Gly Leu Phe Thr His Leu Gly Val Phe Thr Leu Gly
    450                 455                 460

Ala Arg Ala Asp Ser Tyr Tyr Glu Tyr Leu Leu Lys Gln Trp Ile Gln
465                 470                 475                 480

Gly Gly Lys Gln Glu Thr Gln Leu Leu Glu Asp Tyr Val Glu Ala Ile
                485                 490                 495

Glu Gly Val Arg Thr His Leu Leu Arg His Ser Glu Pro Ser Lys Leu
            500                 505                 510
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Thr Phe Val Gly Glu Leu Ala His Gly Arg Phe Ser Ala Lys Met Asp
        515                 520                 525

His Leu Val Cys Phe Leu Pro Gly Thr Leu Ala Leu Gly Val Tyr His
    530                 535                 540

Gly Leu Pro Ala Ser His Met Glu Leu Ala Gln Glu Leu Met Glu Thr
545                 550                 555                 560

Cys Tyr Gln Met Asn Arg Gln Met Glu Thr Gly Leu Ser Pro Glu Ile
                565                 570                 575

Val His Phe Asn Leu Tyr Pro Gln Pro Gly Arg Arg Asp Val Glu Val
            580                 585                 590

Lys Pro Ala Asp Arg His Asn Leu Leu Arg Pro Glu Thr Val Glu Ser
        595                 600                 605

Leu Phe Tyr Leu Tyr Arg Val Thr Gly Asp Arg Lys Tyr Gln Asp Trp
    610                 615                 620

Gly Trp Glu Ile Leu Gln Ser Phe Ser Arg Phe Thr Arg Val Pro Ser
625                 630                 635                 640

Gly Gly Tyr Ser Ser Ile Asn Asn Val Gln Asp Pro Gln Lys Pro Glu
                645                 650                 655

Pro Arg Asp Lys Met Glu Ser Phe Phe Leu Gly Glu Thr Leu Lys Tyr
            660                 665                 670

Leu Phe Leu Leu Phe Ser Asp Asp Pro Asn Leu Leu Ser Leu Asp Ala
        675                 680                 685

Tyr Val Phe Asn Thr Glu Ala His Pro Leu Pro Ile Trp Thr Pro Ala
    690                 695                 700
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/24008

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 23.5, 24.31; 530/300, 350; 435/6, 69.1, 252.3, 320.1, 325

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST, STN (Database: Biosis, Embase, Medline, Scisearch, Lifesci, Caplus)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database GenBank (N_Geneseq_36); Accesion NO: V86545; AGOSTINO et al.; New polynucleotides encoding human secreted proteins-derived from e.g. human blood, kidney, foetal lung, placenta, testes, brain, ovary, pituitary, retina and colon cDNA libraries; 15 OCT 1998; having 100% sequence identity with SEQ ID NO:11. See entire document.	1-7, 21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

25 JANUARY 2001

Date of mailing of the international search report

06 FEB 2001

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Form PCT/ISA/210 (second sheet) (July 1998) ★

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database GenBank (N_Geneseq_36); Accession NO: T23968; MATSUBARA et al.; "Identifying gene signatures in 3'-directed human cDNA library- e.g. for diagnosis of abnormal cell function by preparing cDNA that reflects relative abundance of corresponding mRNA in specific human tissues"; 01 June 1995; having 98.9% sequence identity with SEQ ID NO: 11, see entire document.	1-7, 21
X	Database GenBank (EST); Accession NO:AI92802; NCI-CGAP; "Tumor Gene Index"; 02 September 1999; having 100% sequence identity with SEQ ID NO: 11; Vector: pT7T3D; host cell: DH10B, see entire document.	1-10, 21
X	Database GenBank (EST); Accession NO: H08444; HILLIER et al.; source: clone_lib="Soares infant brain 1NIB"; 23 June 1995; having 98.7% sequence identity with SEQ ID No: 11; Vector: Lafmid BA; host cell: DH10B, see entire document.	1-10, 21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/24008

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-10, 14, 15 and 21, in part

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C07H 21/04, 21/02; C07K 5/00, 14/00; C12Q 1/68; C12N 1/12, 15/63, 15/85, 15/86

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536/23.1, 23.5, 24.31; 530/300, 350; 435/6, 69.1, 252.3, 320.1, 325

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Groups 1-72, claim(s)1-10, 14, 15 and 21, all in part, drawn to an isolated nucleic acid of SEQ ID NO X or a peptide of SEQ ID NO NO: Y, wherein X and Y are values that correlate to those listed in Table 1, and correspond to one of the cDNA Clone Ids, respectively. For example,

If group I is elected, this correlates to Gene no 1, cDNA clone ID HETHR73 of Table 1, wherein X is 11 and Y is 83.

If group 2 is elected, this correlates to Gene No 2, cDNA clone ID HDPFB02, wherein X is 12 and Y is 84.

Groups 73-144, claim(s) 11, 12, 16 and 23, all in part, each group directed to a peptide of SEQ ID NO; Y, wherein Y correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 73 is elected, this correlates to Gene no 1, cDNA clone ID HETHR73 of Table 1, wherein Y is 83.
If group 74 is elected, this correlates to Gene No 2, cDNA clone ID HDPFB02, wherein Y is 84.

Groups 145-216, claim 13, in part, drawn to an isolated antibody which binds to a protein with SEQ ID NO Y, wherein Y correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 145 is elected, this correlates to Gene no 1, cDNA clone ID HETHR73 of Table 1, wherein Y is 83.
If group 146 is elected, this correlates to Gene No 2, cDNA clone ID HDPFB02, wherein Y is 84.

Groups 217-288, claim 17, in part, drawn to a method for preventing, treating or ameliorating an undefined medical condition by administering a polypeptide of SEQ ID NO Y, wherein Y correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 217 is elected, this correlates to Gene no 1, cDNA clone ID HETHR73 of Table 1, wherein Y is 83.
If group 218 is elected, this correlates to Gene No 2, cDNA clone ID HDPFB02, wherein Y is 84.

Groups 289-360, claims 18 and 19, in part, drawn to a method of diagnosis of an undefined pathological condition by determining the presence or absence of a mutation in a polynucleotide of SEQ ID NO X, wherein X correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 289 is elected, this correlates to Gene no 1, cDNA clone ID HETHR73 of Table 1, wherein X is 11.
If group 290 is elected, this correlates to Gene No 2, cDNA clone ID HDPFB02, wherein X is 12.

Groups 361-432, claim 20, in part, drawn to a method of identifying a binding partner to a polypeptide defined by SEQ ID NO Y, wherein Y correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 361 is elected, this correlates to Gene no 1, cDNA clone ID HETHR73 of Table 1, wherein Y is 83.
If group 362 is elected, this correlates to Gene No 2, cDNA clone ID HDPFB02, wherein Y is 84.

Groups 433-504, claim 22, in part, drawn to a method of identifying an activity in a biological assay by identification of the protein in the supernatant wherein the cell expresses a polypeptide encoded by SEQ ID NO X, wherein X correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 433 is elected, this correlates to Gene no 1, cDNA clone ID HETHR73 of Table 1, wherein X is 11.
If group 434 is elected, this correlates to Gene No 2, cDNA clone ID HDPFB02, wherein X is 12.

The inventions listed as Groups 1-504 do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The polynucleotides and polypeptides of each invention are unrelated, each to each other. Where, for example, claim 1, items (e) through (f) do not require a polynucleotide of any degree of specificity to a sequence, it is apparent that Lindeskog et al. (1999, Virology Vol 258(2) 441-450) discloses a DNA encoding a polypeptide wherein said DNA renders claim 1, among the other, not novel. Thus the technical feature of the polynucleotide sequence is not special and the groups are not so linked under PCT Rule 13.1. Additionally the claimed methods produce different products and/or different results which are not coextensive and which do not share the same technical feature.